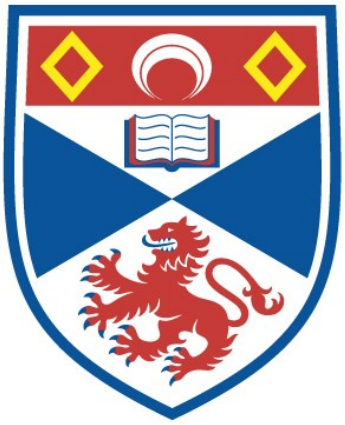
ADIPOSE TISSUE LIPID METABOLISM DURING PREGNANCY AND LACTATION

Keith R. W. Gillon

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1979

SYNOPSIS

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3. Rat adipose tissue lipoprotein lipase activity fell during early-pregnancy, prior to a recovery of enzyme activity in mid-pregnancy. The enzyme activity fell during late pregnancy to very low levels which were maintained until at least day 8 of lactation.

   Lipoprotein lipase activity in mouse adipose tissue fell during early-pregnancy and this low level of activity was maintained until day 17 of pregnancy, when an increase in activity occurred. The increased activity was maintained in early-lactation.

4. The response of rat adipose tissue in vitro to epinephrine stimulation in the release of FFA and glycerol was increased throughout pregnancy and early-lactation. Release of both FFA and glycerol was depressed on day 10 of lactation.

Basal release of FFA increased but not significantly on days 7 and 12 of pregnancy and 3 of lactation. Glycerol release was elevated on day 12 of pregnancy and day 3 of lactation. Evidence is presented that the rate of FFA reesterification in the rat is decreased in early- to mid-pregnancy and increased in late pregnancy.

5. Basal FFA and glycerol release in mouse adipose tissue *in vitro* were not significantly different from controls during pregnancy. FFA release was depressed on day 2 of lactation whereas glycerol release was increased.
   Both FFA and glycerol release in response to epinephrine stimulation increased, but not significantly, in late-pregnancy and markedly increased in early lactation.

6. Prolactin injections had no significant effect on virgin rat adipose tissue lipoprotein lipase activity *in vivo*. Oestradiol benzoate markedly depressed lipoprotein lipase activity in virgin rat adipose tissue *in vivo*, and simultaneous administration of oestradiol benzoate plus prolactin did not decrease enzyme activity further. Simultaneous administration of oestradiol benzoate and α-ergocryptine produced a significant decrease in lipoprotein lipase activity in virgin rat adipose tissue *in vivo*.

7. α-Ergocryptine administration to lactating rats reduced litter weight gain and increased the activity of lipoprotein lipase in adipose tissue *in vivo*.

ADIPOSE TISSUE LIPID METABOLISM DURING PREGNANCY AND LACTATION.

A thesis submitted to the University of St. Andrews by

KEITH R.W. GILLON

for the degree of Doctor of Philosophy.

March 1979.

DECLARATION.

I hereby declare that this thesis is a record of work done by me, and composed by me, and to the best of my knowledge has not previously been presented for a higher degree. This research was carried out in the Department of Biochemistry in the University of St.Andrews under the supervision of Dr. C. R. Strong.

KEITH R.W. GILLON.

CERTIFICATE

I hereby certify that Keith R.W. Gillon has been engaged in research work under my direction, that he has fulfilled the conditions of Ordinance No. 12 and that he is qualified to submit the accompanying thesis for the Degree of Doctor of Philosophy.

C.R.Strong

CONTENTS

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to Hazel

and to my Parents.

1. INTRODUCTION.

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For many years it was thought that adipose tissue was a relatively inactive tissue in body function. However, it is now known that adipose tissue plays a vital role in the regulation of mammalian energy balance. This is particularly evident during times of metabolic stress, e.g. starvation or prolonged muscular exercise.

One such period of stress is that during late pregnancy and lactation when the female rat must provide a supply of nutrient for the foetuses and, later, the pups. There is a flow of nutrient to the placental unit during pregnancy when the foetuses are developing and also provision of substrates, carbohydrates, amino acids and lipid, to the mammary glands for milk synthesis (1). Thus, in the synthesis of milk, the mammary gland has a well defined role in the reproductive cycle, but it is not known to what extent other tissues contribute material for foetal and pup development.

Studies by Graziano (2) showed that as mammary tissue develops through its underlying fat pad during pregnancy, the amount of adipose tissue is reduced until, in the lactating animal, the adipose tissue content of the gland is very small. It is not known if this results from both decreased synthetic activity and increased mobilization of adipose tissue depot fat. Since the adipose tissue content of the mammary glands is reduced during late pregnancy and lactation, it is possible that some, or all adipose

tissue lipid stores in the body undergo mobilization and exhibit diminished lipid synthetic activity at this time to provide nutrient for uptake by the mammary glands. In the mouse, histological studies have shown that both the mammary fat pad and parametrial fat pad are depleted of lipid during late pregnancy and lactation, that the depletion is dependent on the number of nursing young, and that there is replenishment of depleted fat cells after weaning (3). Direct evidence also suggests that adipose tissue may be involved in nutrient supply during pregnancy and lactation. Food intake in the rat increases during pregnancy (4) and this results in increased body fat (5, 149). Increased lipid synthetic activity during early pregnancy followed by depressed activity during late pregnancy and lactation has been reported for adipose tissue in the rat (24). In the mouse, in parturitions after the first, greater amounts of fat were found in pregnant animals compared with controls (150).

According to Spray (5) the increased body fat is lost during lactation in the rat but not in the mouse, although it was later shown that mice at the end of lactation had less fat than controls (150). Mobilization of adipose tissue lipid is elevated in the rat during lactation (7), and Elliot has shown increased lipolytic response of fat cells of late pregnant women compared with cells from women non-pregnant or 1-3 days post-partum (8).

It has been suggested that there is a biphasic pattern of lipid synthetic activity by adipose tissue during pregnancy (4). It is thought that adipose tissue lipid stores are built up during early and mid-pregnancy in preparation for mobilization during the later stages of pregnancy and lactation. Since these studies were confined to a few selected days in pregnancy or lactation

it was decided to build-up a more detailed picture of adipose tissue lipid metabolism during pregnancy and lactation. The results are reported later in this thesis. It may then be possible to assign a role in the provision of nutrient for milk synthesis to adipose tissue.

Before discussing the work done on adipose tissue metabolism during the reproductive cycle, it is perhaps worthwhile considering general carbohydrate and lipid metabolism in rat adipose tissue. This will be limited to discussion of those aspects of metabolism most relevant to this thesis. Much of the early work to elucidate this was done using epididymal adipose tissue (9, 10, 11). A brief scheme is presented in Figure 1.

1.1. Glucose oxidation in adipose tissue

Adipose tissue takes up and oxidizes glucose, the transport process being sensitive to the action of insulin (12). Glucose oxidation proceeds via the routes of glycolysis and pentose phosphate pathway in roughly equal proportions (20). In the glycolytic pathway an intermediate stage is the production of the three-carbon units glyceraldehyde-3-phosphate and di-hydroxy acetone phosphate (G-3-P and DHAP respectively). Adipose tissue contains relatively small amounts of glycerokinase activity (14), and it is thought that the fate of part of the DHAP produced in glycolysis is production of sn-glycerol-3-phosphate via glycerol phosphate dehydrogenase. This is then condensed with fatty-acyl CoA derivatives to form triglycerides. G-3-P is further metabolized in glycolysis to form pyruvate, which, by the action of pyruvate

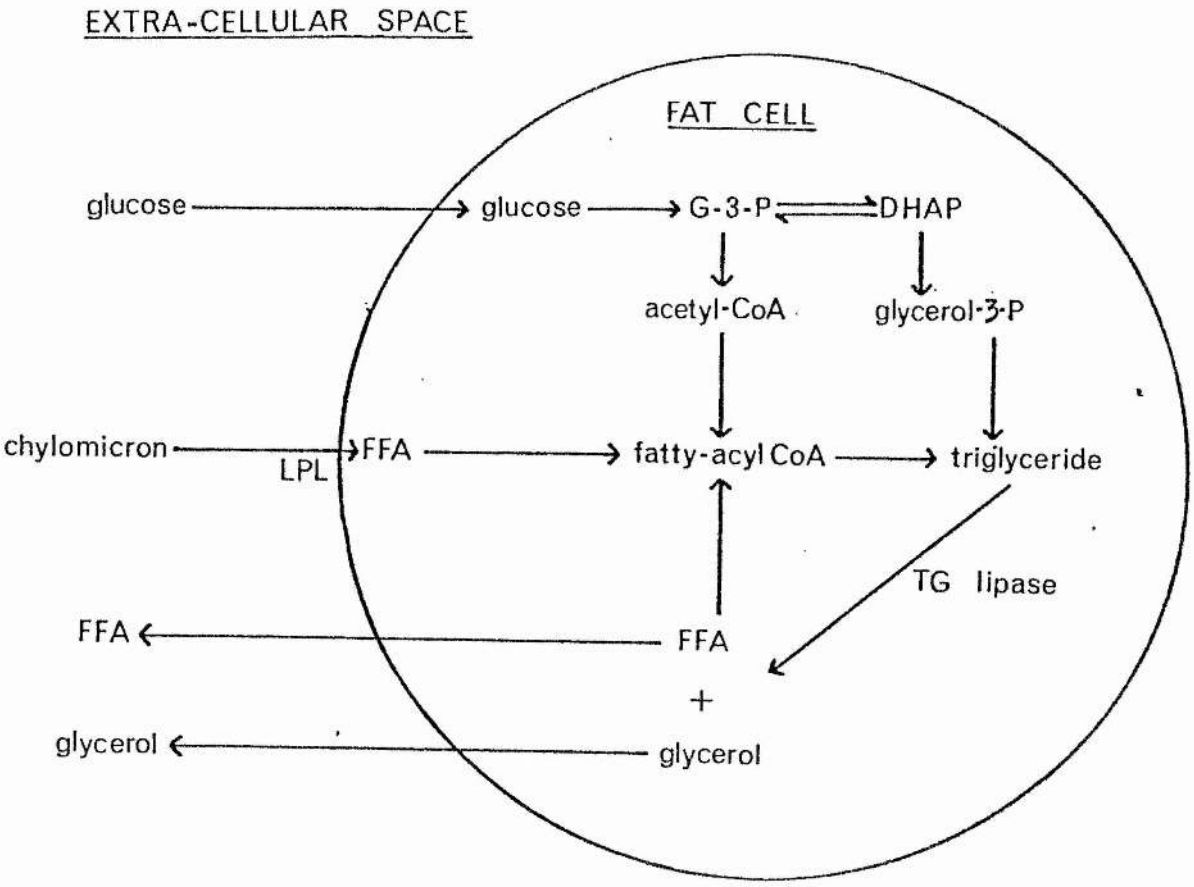


Figure 1. General scheme of glucose and lipid metabolism in adipose tissue.

dehydrogenase, is converted to acetyl-CoA. This enters the tricarboxylic acid cycle and is oxidized with, ultimately, production of ATP. Acetyl-CoA also provides the two carbon precursors for the synthesis of long chain fatty acids. The pentose phosphate pathway is also a source of three carbon units which may be used as described above. During oxidation of glucose-6-phosphate via the pentose phosphate pathway, NADP is converted to NADPH which is the nucleotide involved in the reduction steps of fatty acid synthesis. Thus adipose tissue has the capability to produce the necessary substrates for fatty acid synthesis. Significant fatty acid synthesis does in fact take place in adipose tissue (13).

1.2. Fatty acid biosynthesis in adipose tissue

Fatty acid synthesis in adipose tissue takes place in a similar way to that described for rat mammary gland, i.e. the malonyl-CoA pathway (15). The enzymes responsible for de novo fatty acid synthesis exist in the form of a multi-enzyme complex known as fatty acid synthetase (FAS). Also contained in the complex are binding sites for acetyl- and malonyl-CoA. Binding of acetyl-CoA is the first stage in synthesis. Acetyl-CoA is synthesized in adipose tissue from glucose via glycolysis. Glucose is converted to pyruvate via intermediates and pyruvate is converted to acetyl-CoA by the pyruvate dehydrogenase complex. The individual enzymes involved in the complex are pyruvate dehydrogenase which catalyses decarboxylation of pyruvate; dihydrolipoyl transacetylase which transfers the acetyl group to coenzyme A to yield acetyl-CoA; and dihydrolipoyl dehydrogenase which regenerates the oxidized form of dihydrolipoyl transacetylase necessary for its activity. Acetyl-CoA is transferred from a loading site via a freely moving central thiol group (4'-phosphopantetheine) to a

peripheral thiol group (cysteine). Malonyl-CoA, formed by reaction between acetyl-CoA and CO_2, then binds to the central thiol via the loading site. Condensation of acetyl- and malonyl-CoA occurs with loss of CO_2. The product of this reaction undergoes reduction, dehydration and a further reduction. The reducing nucleotide is NADPH, the majority of which (ca. 60%) comes from the oxidation of glucose via the pentose phosphate pathway (157). Following the second reduction, another molecule of malonyl-CoA binds to the complex, and the process is repeated. The final product of this pathway in mammal is largely palmitic acid, unlike the bacterial and yeast FAS which produces palmityl-CoA (16).

Conversion of pyruvate to acetyl-CoA takes place intra-mitochondrially and fatty acid synthesis in the cytosol. Since the mitochondrial membrane is only sparingly permeable to CoA derivatives, alternative methods for supply of acetyl-CoA to the cytosol have been suggested (17). Possible mechanisms include deacylation of acetyl-CoA to acetate which is freely permeable across the mitochondrial membrane, conversion to acetyl-carnitine, and, most likely, the condensation of acetyl-CoA and oxaloacetate to form citrate (18, 158). Citrate crosses the mitochondrial membrane to the cytosol where it is cleaved by ATP-citrate lyase. After cleavage of citrate the oxaloacetate produced is converted to malate by NAD-linked malate dehydrogenase and the malate oxidized by NADP-linked malate dehydrogenase. The pyruvate formed may then re-enter the mitochondrion and is converted to oxalo-acetate via pyruvate carboxylase. The oxidation of malate is considered to be the source of the remainder of the NADPH required for the reduction steps of fatty acid synthesis (19).

Adipose tissue is sensitive to the action of insulin and it has been demonstrated that both glucose oxidation and fatty acid synthesis

rates are increased by the addition of insulin, the level of stimulation being dose dependent (13, 20, 21). Insulin promotes the transport of glucose into the fat cell (22), and it is thought that the intracellular concentration of glucose is the regulator of the activity of hexokinase, the first enzyme involved in glucose oxidation. It is possible that insulin also exerts control over the activities of pyruvate dehydrogenase and acetyl-CoA carboxylase (23). Insulin therefore increases fatty acid synthesis by increasing the flow of glucose through glycolysis. This releases NADP which increases the flow of glucose through the pentose phosphate pathway, producing NADPH for use in fatty acid synthesis. The rate of synthesis of triglyceride by adipose tissue may therefore be regulated by the level of circulating insulin.

1.3. Glucose oxidation and fatty acid synthesis during the rat reproductive cycle.

Several workers have studied the effect of pregnancy and lactation on the utilization of glucose and the rate of fatty acid synthesis in rodent adipose tissue. Smith observed small increases in glucose utilization from 1-^{14}C - and 6-^{14}C -glucose by rat parametrial adipose tissue on days 7 and 16 of pregnancy (24). Knopp showed a decreased glucose conversion to $^{14}CO_2$ from 6-^{14}C - glucose but not 1-^{14}C -glucose in lumbar fat pads from 19 day pregnant rats compared with virgin animals (25).

Incorporation of glucose into fatty acids is reduced on day 20 of pregnancy and lactation (24), and depressed rates of fatty acid synthesis from U-^{14}C -glucose in late pregnancy have been shown following increased fatty acid synthesis in mid-gestation (4).

Fain and Scow observed increased fatty acid synthetic activity during early and mid-pregnancy followed by decreased rates in late pregnancy (6).

1.4. Lipoprotein Lipase Activity of Adipose Tissue.

During periods of positive energy balance, with an ample supply of glucose and lipid, mammalian adipose tissue is active in the removal of triglyceride from the circulation (26, 27). Mobilization of lipid from adipose tissue at this time is minimal (28). In contrast, during starvation, the loss of weight by the animal is accompanied by depletion of the adipose tissue depots. Adipose tissue from such animals in vitro shows elevated rates of release of FFA and glycerol i.e. increased lipid mobilization (29). Diabetes, when glucose utilization is decreased and lipid utilization is increased by tissues, is also characterized by decreased uptake of lipid by adipose tissue (30). These studies showed that the activity of lipoprotein lipase in adipose tissue is elevated in periods of positive energy balance and decreased during starvation and diabetes (30-33). Refeeding starved animals returns lipoprotein lipase activity in adipose tissue to levels similar to or above normal, further evidence for the involvement of the enzyme in the storage of lipid in adipose tissue (31). Lipoprotein lipase activity has also been demonstrated in other tissues (34) and the activity appears to be altered with different physiological conditions e.g. starvation and lactation (35). Thus lipoprotein lipase is the enzyme which controls the uptake of blood lipid by tissues.

1.4.1. Site and Mechanism of Action of Lipoprotein Lipase

Dietary triglyceride in the gut is absorbed through the intestinal wall and absorbed into the bloodstream. Pancreatic lipase appears to act by hydrolysing triglyceride in the gut mainly to FFA and monoglyceride which, following micelle formation, pass into the epithelial cells by passive diffusion. In the epithelial cells FFA and monoglycerides are reesterified to triglyceride. The triglyceride is then incorporated into lipoproteins known as chylomicra which are almost totally composed of a triglyceride core surrounded by mainly a phospholipid and protein coat. It is in this form that triglycerides are transported via the lymph into the bloodstream. Liver and adipose tissue absorb about 30% each of the triglyceride which enters the bloodstream (35). The liver hydrolyses triglyceride to obtain FFA for oxidation and those not oxidized are re-incorporated into phosphatides, cholesterol esters and triglycerides which re-enter the bloodstream as very low density lipoproteins (VLDL), which may be utilized by extrahepatic tissues (36). This study also showed that by injecting doubly-labelled triglycerides into rats, very little of the labelled glycerol backbone was found in adipose tissue. This suggests that hydrolysis of triglyceride by adipose tissue and other tissues is necessary for uptake of the fatty acid moieties of the triglyceride, and that glycerol is not utilized by adipose tissue to a significant extent.

It was demonstrated that addition of heparin to incubations of adipose tissue from fed rats increased the lipolytic activity in the medium (31). Lipoprotein lipase is also released into the circulation following injection of heparin into rats (36, 37). The site of

action of lipoprotein lipase is therefore thought to be the endothelial cells of the capillaries supplying adipose tissue. Much of the evidence for the site of action of lipoprotein lipase is indirect, but more recently Scow (38) has produced electron microscopical evidence. It is proposed that when chylomicra reach rat adipose tissue they are surrounded by finger-like projections from the capillary endothelial cells. There is little evidence that the chylomicra penetrate the endothelium. This suggests that enzymic action takes place in the endothelial cells or on the endothelial luminal surface. Indeed during the passage of chylomicra into the endothelial cells, grains of electron dense material can be seen which are presumed to be products of enzymic degradation of the chylomicra. It appears then that triglyceride is hydrolysed in or near the endothelial cells and that the FFA produced is transported to the fat cells via a series of vacuoles and vesicles. Glycerol is not absorbed into or utilized by the fat cells, consistent with the lack of glycerokinase activity in adipose tissue.

1.4.2. Properties of Lipoprotein Lipase.

Lipoprotein lipase has been characterized. It is found in tissues which utilize triglyceride and has an apparent molecular weight of 72600 (39). High molecular weight estimates were, however, recently considered to be artifactual due to association of the enzyme with particulate material (151). It is activated by a serum lipoprotein fraction (40-42), is inhibited by 0.5N NaCl and fluoride and has a pH optimum of 8.0 - 8.5 (43). Evidence shows that the enzyme exhibits substrate specificity, having preference for the hydrolysis of the position 1 primary ester bond (40, 44). It will hydrolyse triglyceride and 1,2(2,3)-diglyceride to 2-monoglyceride, and 1(3)-monoglyceride

to glycerol, but has no effect on 2-monoglyceride. Isomerization of 2-monoglyceride to 1(3)-monoglyceride results in complete hydrolysis.

Column chromatography of adipose and heart tissue protein extracts has shown the existence of two enzyme species with similar pH optima and characteristics (41, 45). The enzyme species have been named LPLa and LPLb and both exhibit Lipolytic properties. Post-heparin plasma shows only one form of lipoprotein lipase and by chromatographical studies it is most closely related to LPLa (41). These forms of the enzyme are affected by nutritional status (45). The activity of LPLa and LPLb is decreased in adipose tissue and increased in heart tissue from starved rats and these effects are reversed by refeeding. Post-heparin plasma lipoprotein lipase activity is unaffected by nutritional status.

1.4.3. Release of Lipoprotein Lipase from Fat Cells

Release of LPLa from rat adipose tissue in vitro to the medium proceeds linearly for at least 45 minutes when glucose is present in the medium (46). Heparin in the medium will also induce release of LPLa (41). The transport process of LPLa is energy requiring and will only take place maximally in the presence of glucose, Ca^{++} ions, K^{+} ions and albumin (46, 47). When cycloheximide is present in the medium, incorporation of amino acids into fat cell protein is inhibited by over 90% (46). Under these conditions release of LPLa from rat adipose tissue to the medium in the presence of glucose continued unaffected, and shows that release of LPLa is independent of protein synthesis. It is suggested that the increase in LPLa in the medium is brought about by activation of existing enzyme and not by de novo enzyme synthesis. Current theory is that

LPLb is the intracellular, constitutive form of the enzyme and that LPLa is the inducible form which is active in triglyceride hydrolysis at the capillary endothelium. It is thought that heparin and albumin may be involved in the conversion of LPLb $\longrightarrow$ LPLa, and in the transfer of LPLa to its site of action (41, 46).

1.4.4. Effect of Hormones on the Activity of Lipoprotein Lipase

Hypophysectomy of lactating rats causes an increase in lipoprotein lipase activity in adipose tissue and a decrease in activity in mammary gland (48). Injection of prolactin into these animals blocked the increase in activity in adipose tissue and increased the activity in mammary gland.

Desai (49) showed that insulin + glucose produced an increase in enzyme activity in adipose tissue *in vitro* from fasted rats and maintained the activity in adipose tissue from fed rats. This confirmed the results of Borensztajn (50). Since glucose is known to induce LPLa (46), insulin activation may be due to its effect on glucose entry to adipose tissue. This is possible since the rise in activity in adipose tissue from fasted rats caused by insulin (49) was blocked on administration of blockers of protein synthesis. Glucose is known to induce protein synthesis in the fat cell (46).

Oestrogen treatment of rats depresses adipose tissue lipase activity and causes a small increase in activity in mammary gland (51-53). Progesterone has been reported to increase the activity of adipose tissue lipoprotein lipase (53, 54). Spooner (54) also showed that injected $PGF_{2\alpha}$ increased the activity of lipoprotein

lipase in mammary gland and reduced the activity of the enzyme in adipose tissue from 20 day pregnant rats.

Summary

i. Prolactin increases the activity of lipoprotein lipase in mammary gland and blocks the rise in enzyme activity in adipose tissue of hypophysectomized rats.

ii. Insulin + glucose increases lipase activity in adipose tissue of fasted rats and maintains activity in tissue from fed rats.

iii. Oestrogen depresses lipase activity in rat adipose tissue.

iv. Progesterone increases lipase activity in rat adipose tissue.

v. $PGF_{2\alpha}$ increases lipase activity in mammary gland and reduces that in adipose tissue.

1.4.5. Lipoprotein Lipase Activity during Pregnancy and Lactation

Much of the fatty acid secreted in rat milk is derived from dietary triglyceride and so the type of fatty acid in milk is partly dependent on the diet (55). It is thought that control of the uptake of blood lipid into mammary gland is dependent on the activity of lipoprotein lipase. Since the lipid synthetic activity of adipose tissue declines during late pregnancy and lactation (4, 24) it is important to determine if lipid uptake by this tissue is also diminished at this time, thus re-routing lipid to mammary gland for milk triglyceride synthesis. The concentration of circulating blood triglyceride reaches a peak in the rat a few days before parturition followed by a rapid decline prior to parturition (56, 57, 152). It was also demonstrated that the high activity of lipoprotein lipase

in adipose tissue during mid-gestation falls to a low level by day 21 of pregnancy (57). Otway (56) proposed that the lipaemia of pregnancy is due to the low activity of adipose tissue lipoprotein lipase and the disappearance of this lipaemia is brought about by the increase in mammary gland lipoprotein lipase activity at parturition. This rise in mammary gland lipase activity has been described for various species (57-62). Plasma triglyceride remains at low levels during lactation, due partly at least to the increase in mammary gland lipoprotein lipase activity which occurs at parturition (57).

It appears, therefore, that by decreasing the activity of lipoprotein lipase in adipose tissue (parametrial, perirenal and subcutaneous and so probably all adipose tissue depots of the body (56, 57)), the mammal may re-route dietary lipid to mammary gland for milk synthesis.

1.5. Lipolysis in Adipose Tissue.

In the mammal during times of positive energy balance glucose is the preferential source of energy for the tissues. Some physiological conditions result in stored carbohydrate being insufficient for the needs of the tissues or, as in diabetes, unavailable for utilization. Since tissue lipid stores are limited, an external source of lipid is necessary. Elevated levels of blood FFA, derived from adipose tissue, have been reported during starvation (29) and diabetes (63). The mammal is thus capable of mobilizing lipid reserves in times of carbohydrate deprivation as an alternative fuel supply to glucose.

Since the permeability of the fat cell membrane to triglyceride is very low, hydrolysis of the stored triglyceride is necessary for mobilization. It has been demonstrated that FFA levels in blood are elevated during starvation and the presence of glycerol in the medium from adipose tissue of fasted rats *in vitro* has been reported (29). This is the process of lipolysis and release of FFA and glycerol from adipose cells appears to proceed by passive diffusion.

1.5.1. Triglyceride Lipase of Lipolysis

Since the site of action of lipoprotein lipase is the luminal surface of the capillary endothelium, this enzyme is not responsible for intracellular hydrolysis of adipose tissue triglyceride during lipolysis. The lipase responsible for this was described by Rizack (64) and Gorin (65). The enzyme differs from lipoprotein lipase in that it has a pH optimum of 6.8, is not inhibited by NaCl and requires the presence of phosphate, albumin and triglyceride for full activity *in vitro*. It has a minimum molecular weight of 86000 and exhibits tri-, di-, and monoglyceride lipase activities in the ratio 1:10:15 (66). The need for albumin in the medium is consistent with its physiological role. Due to their hydrophobic nature, FFA must be transported in plasma combined with a carrier protein, albumin. Release of FFA to the incubation medium is greatly increased by addition of albumin (67), and it is possible that removal of FFA in this way further stimulates the enzyme. The theoretical value for the ratio of release of FFA and glycerol is three. However, when adipose tissue is oxidizing glucose

concomitantly, FFA may be re-esterified in reaction with sn-glycerol-3-phosphate produced via glycolysis. This reduces the ratio of release of FFA and glycerol. This, and the fact that adipose tissue cannot re-utilize glycerol (14), has led to the acceptance of glycerol release as the true index of lipolysis *in vitro*.

The exact mechanism of activation of triglyceride lipase (TG lipase) has not been fully elucidated. It may be regulated by a phosphorylation/dephosphorylation mechanism similar to that reported for muscle glycogen phosphorylase (66, 68). From studies which show effects on enzyme activity *in vitro* by a protein-kinase, ATP-Mg^{++} and cAMP (66, 69), it is thought that the lipase is regulated via the adenyl cyclase system where cAMP is the secondary messenger of hormone action on the fat cell (Figure 2). It has been demonstrated that the ability of the enzyme to hydrolyse triacylglycerols *in vitro* is greatest when protein kinase, ATP-Mg^{++}, albumin and cAMP are present. It is thought that activation of receptors on the fat cell membrane by hormones stimulate adenyl cyclase to produce cAMP (3', 5' cyclic adenosine monophosphate). This activates a protein kinase which, in turn, activates the lipase by phosphorylation. In the muscle glycogen phosphorylase system, a protein kinase phosphorylates a phosphorylase kinase which, in turn, activates the glycogen phosphorylase. Belfrage (66) indicates that no second kinase enzyme is involved in activation of TG lipase. By altering the activity of adenyl cyclase and 3', 5' phosphodiesterase, which control the level of cAMP in adipose tissue, regulation of the lipolytic rate is possible. Recent investigations have shown that the

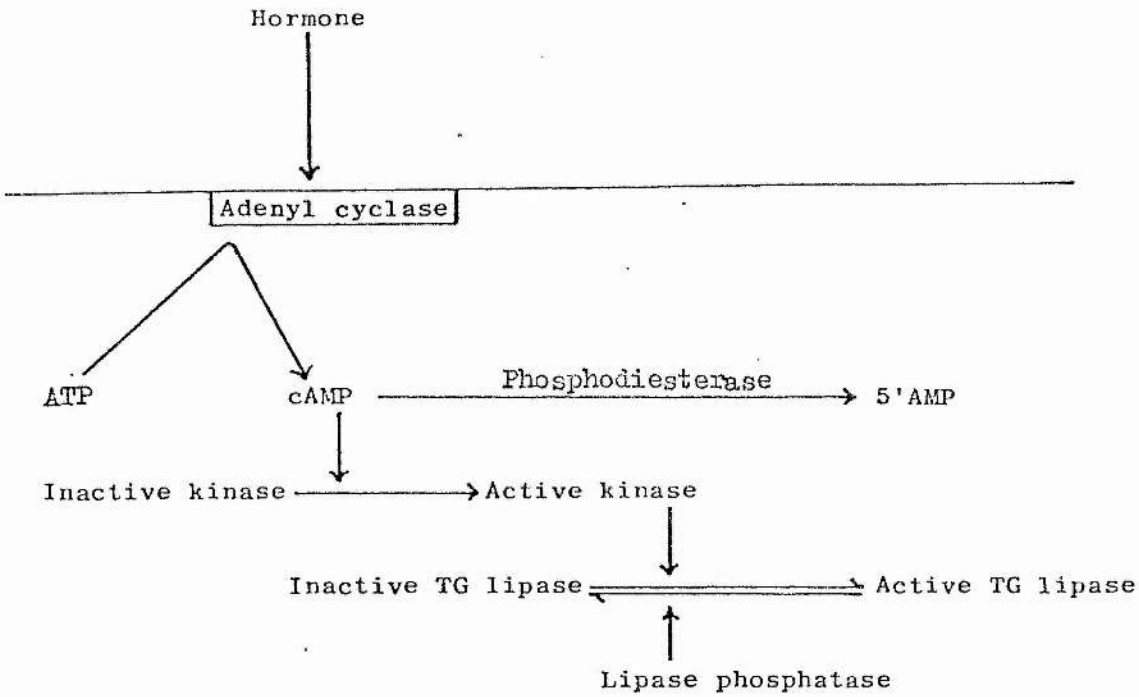


Figure 2. Scheme of hormone-stimulated activation of adipose tissue triglyceride lipase.

rise in cAMP is transient and may be involved only in the initial stimulation of protein kinase (69). It is not known how this increased kinase activity is maintained, particularly since a second stimulation of fat cells in vitro does not result in a second rise in cAMP levels although the lipolytic rate is elevated (69). However, cAMP may be compartmentalized or in different pools which may be altered independently of the total cAMP level (69-71). Severson et al (68) have shown that deactivation of triglyceride lipase requires Mg^{++} ions and is inhibited by phosphate, consistent with deactivation of the enzyme by a lipase phosphatase.

1.5.2. Hormones in Lipolysis

TG lipase is affected by a number of hormones which has led to its designation as the 'hormone-stimulated lipase'. Epinephrine and nor-epinephrine are strongly lipolytic at physiological concentrations (0.1μg/ml), and stimulation in vitro is dose dependent (69, 72). Solomon (73), using a system of fat-cell perifusion, has demonstrated the lipolytic effect of epinephrine and its ability to restimulate fat cells following a period of perifusion with buffer alone. Stimulation of the nerves supplying adipose tissue increases the lipolytic rate, due most likely to the release of nor-epinephrine at the nerve endings (19). Mobilization of lipid during times of stress, either psychological or physiological, is thought to be caused partly by release of nor-epinephrine and epinephrine.

During stress there is release of ACTH which has been shown to be a potent lipolytic agent (29, 74). Hypophysectomy of rats

results in decreased fat mobilization and reduced sensitivity of adipose tissue from such animals to epinephrine. If adipose tissue from hypophysectomized rats is pre-incubated for 3 hours with dexamethazone prior to stimulation of the tissue with low concentrations of epinephrine, sensitivity of the tissue to epinephrine is increased (75). There was a lag period of more than 2 hours in this increase. If actinomycin D is added to the medium during the pre-incubation period, the increase in glycerol release in response to epinephrine does not occur. It is suggested that dexamethazone may induce cAMP production secondary to induction of synthesis of new protein. Furthermore, Paoletti and Smith (communication to Vaughan (13)) have shown that the effects of ACTH on FFA release are not observed in adipose tissue depleted of catecholamine. ACTH may release norepinephrine stored in the tissue and this produces the lipolytic effect attributed to ACTH.

Vaughan (76) has demonstrated that growth hormone, glucagon and TSH also show lipolytic activity. Indeed the thyroid gland itself appears to be important in 'in vivo' lipolysis since fat cells of hypothyroid rats are unresponsive to stimulation by epinephrine or glucagon (154, 155, 156). Triiodothyronine treatment of these animals does in fact restore the lipolytic response of fat cells to epinephrine (156).

Insulin, unlike the other hormones, is antilipolytic at low concentrations of epinephrine (0.1µg/ml), but it does not appear to be antilipolytic at maximal concentrations of epinephrine; in fact, lipolysis is further enhanced by insulin under these conditions. Insulin may react with more than one type of receptor on the fat cell membrane (79). Alternatively, the different effects of insulin

may be the result of various levels of saturation of the same receptor (79). Insulin appears to act as an antilipolytic agent by lowering the level of cAMP intracellularly by increasing the activity of cAMP phosphodiesterase (49).

1.5.3. The Effect of Pregnancy and Lactation on Lipolysis in Adipose Tissue

Evidence, both direct and indirect, suggests that mobilization of adipose tissue triglyceride may occur during late pregnancy and lactation. Elias (3) demonstrated, by histological studies, that the mouse parametrial fat pad is depleted during lactation and that the extent of depletion is dependent on the number of nursing young and length of lactation. However, the weight of the rat parametrial fat pad does not alter significantly during pregnancy (57). The pad was not weighed during lactation.

The content of glycerol and FFA in tissue and medium during incubation of parametrial adipose tissue from non-pregnant, 2 and 14 day lactating and involuting rats has been measured (7). Data shows that there is an increased content of FFA and glycerol in adipose tissue during lactation. Both basal and epinephrine-stimulated release of FFA and glycerol into the medium are increased using adipose tissue from lactating rats compared with tissue from unmated animals. Lipolysis during pregnancy was not included in this study but Elliot (8) has quantitated lipolytic rates in adipocytes from non-pregnant, late pregnant and lactating women. It was found that lipolytic response of the cells to epinephrine was increased in late pregnant versus non-pregnant or 3-day lactating women. Knopp et al (25) have also demonstrated increased net lipolysis in adipose tissue from fed 19-day pregnant rats compared with virgins.

It appears that mobilization of adipose tissue lipid does occur during lactation in the mouse, rat and human but the timing and control of the lipolytic stimulus is not known.

1.6.1. Hormone Levels during Pregnancy in the Rat

Clearly many factors influence adipose tissue metabolism. Since the tissue is responsive to a number of hormones, it is possible that the main control mechanism is hormonal. It is necessary, therefore, in attempting to explain changes in adipose tissue metabolism, to determine changes in circulating hormone levels throughout pregnancy and lactation (Plate 1).

Progesterone levels are elevated during pregnancy reaching a peak at days 15 and 16, and thereafter declining to very low levels at parturition (80). Progesterone levels are also elevated from day 4 until day 12 of lactation and thereafter decline (81).

Oestrogen levels increase gradually from day 11 until day 19 of pregnancy, followed by a rapid increase which is maintained until parturition (82). No significant level of oestrogen was detected the day after parturition (83).

The level of the pituitary hormone LH is increased during the first half of pregnancy (80), consistent with the proposition that it is responsible for progestin production at this time (84). After day 12 the level remains very low prior to a sharp rise before parturition.

Prolactin levels are very low throughout pregnancy and then rise very sharply on day 21. This elevated level is maintained throughout lactation (80, 85, 86).

Plate 1. Serum Hormone Levels during Pregnancy in the Rat.

Abbreviations:

P : Progesterone ————

PRL : Prolactin ————

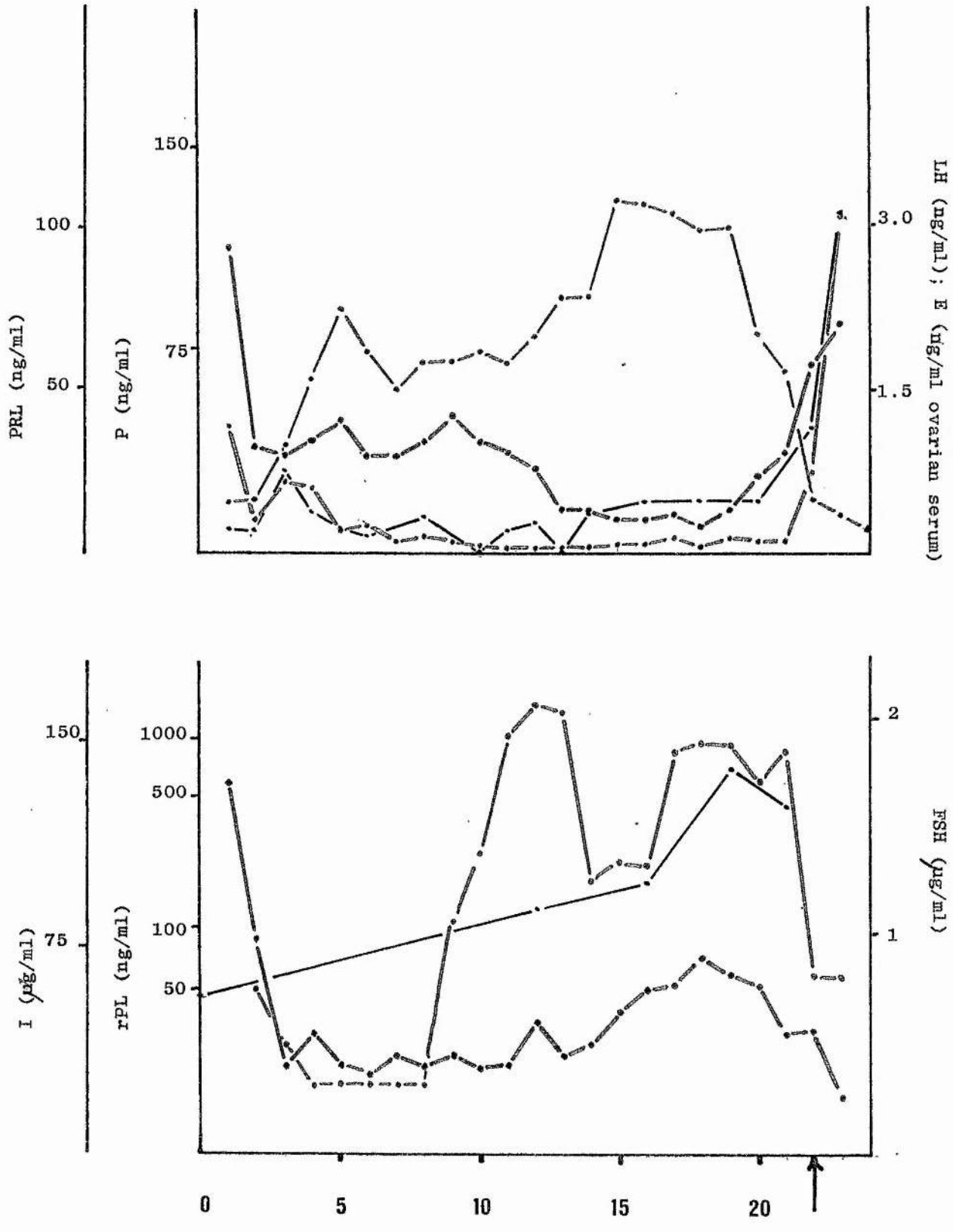
LH : Luteinizing hormone ————

E : Estradiol ————

rPL : Rat placental lactogen ————

FSH : Follicle stimulating hormone ————

I : Insulin ————


PRL (ng/ml)
P (ng/ml)
LH (ng/ml); E (ng/ml ovarian serum)
150
100
75
50
3.0
1.5
I (µg/ml)
rPL (ng/ml)
FSH (µg/ml)
150
75
1000
500
100
50
2
1
0
5
10
15
20
Days of Pregnancy.

FSH levels are low from day 2 until day 15 of pregnancy. There is then a small rise which is maintained until parturition (85).

Insulin concentrations are elevated on day 12 of pregnancy and remain so until day 21 (4). Similarly corticosteroid levels increase slowly from day 12 of pregnancy followed by a sharp rise at parturition (87).

Rat placental lactogen levels have recently been quantitated during pregnancy (88). Significant concentrations of placental lactogen appear on day 9 of pregnancy followed by a peak on day 13. The levels of placental lactogen decrease between days 14 and 16, prior to a rise on day 17 which is maintained until the last day of pregnancy. The hormone is rapidly removed before parturition.

1.6.2. Possible involvement of Hormones in the control of rat pregnancy

A theory for the hormonal control of pregnancy in the rat has been proposed by Raj (84). Following fertilization the follicles of the cycling rat must be converted to the functional, progesterone-secreting corpora lutea of pregnancy. This is thought to be brought about by elevated levels of LH and prolactin a few hours after coitus. The pituitary is necessary during the first 12 days of pregnancy (84, 89), and it is proposed that LH is the luteotropic factor at this time. Neutralization of LH by LH antiserum after day 12 has no effect on pregnancy maintenance (81) and is therefore not the luteotropin during the second half of pregnancy. Both LH and prolactin have been shown to stimulate ovarian progesterone synthesis

(90, 91). In hypophysectomized rats, prolactin and oestrogen injections are most effective in bringing about implantation and cause most of the animals to maintain their pregnancies (89, 92). It was thought that prolactin might be the luteotropin of late pregnancy. However, prolactin doses as high as 10 mgs. are required in vitro to stimulate secretion of progesterone from rat ovaries (91). Since serum prolactin concentrations are very low until two days prior to parturition, it is unlikely that this hormone acts as the luteotropic stimulus. Shiu (88) has demonstrated that rat placental lactogen increases from day 12 and remains elevated until the time when progesterone is removed. Indeed injection of antibodies to hPL into rats between days 11 - 16 of pregnancy and day 16 to term resulted in increased foetal resorption and later pup death due to impairment of the ability of the mothers to lactate (93).

It is possible that LH release is reduced due to negative feedback to the hypothalamus by oestrogen and progesterone which reach critical levels on day 12. Thereafter placental lactogen may be the luteotropic factor. It is possible that prolactin or placental lactogen simply maintains LH-stimulated progesterone output since prolactin inhibits the conversion of progesterone to its derivative 20 - hydroxy pregn-4-en-3-one (94, 95).

A marked decline in circulating progesterone is a prerequisite for parturition in the rat (96). Horton (97) has suggested that this is brought about by the production of $PGF_{2\alpha}$ by the uterus or placenta. $PGF_{2\alpha}$ is a potent luteolytic agent in a number of species.

1.7. Hormonal control of lactogenesis

The hormones responsible for the control of mammary gland development and milk synthesis are also important in discussing the control of adipose tissue metabolism during late pregnancy and lactation.

Ceriani (98, 99), using hypophysectomized-adrenalectomized-ovariectomized rats, and Talwalker (100), using rat foetal mammary gland in organ culture, have outlined the hormones required for mammary gland development. Insulin is necessary for stimulation of growth and lumen formation in ducts, and prolactin enhances this growth response. Aldosterone induces ductule development and the appearance of secretion. Progesterone further increases the secretory response. However, it seems that for full *in vitro* lobuloalveolar development insulin, oestrogen, progesterone, adrenal hormones, prolactin and growth hormone are necessary (101, 102).

Hallowes *et al.* (103) demonstrated that insulin, corticosterone and either prolactin or growth hormone induced greater fatty acid synthesis in mammary gland explants of 10-day pregnant and 5-day lactating rats. Insulin, corticosterone and prolactin induced increases in lipid synthesis in mammary explants from mid-pregnant mice (104). Casein synthesis by explants from virgin and pregnant rats was increased by insulin, corticosterone plus either prolactin or growth hormone (105). Thus, the hormonal combination which brings about milk production in the mouse and rat mammary glands appears to be insulin, corticosterone, prolactin and/or growth hormone.

1.8. Aims of this Study

Since lipid metabolism in adipose tissue has been studied in the main on only a few selected days of pregnancy and lactation, it was decided to build up more comprehensive data of synthesis, uptake and release of lipids by rat and mouse parametrial adipose tissue. It was hoped that this would allow conclusions to be made concerning the role of the tissue in nutrient supply during the stress of pregnancy and lactation.

More specifically, if previous results concerning lipoprotein lipase activity during pregnancy and lactation were confirmed (56, 57), it was hoped to attempt to elucidate some aspects of hormonal control of the activity of the enzyme at this time.

2. MATERIALS AND METHODS

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2.1. Materials

U-^{14}C -glucose, 1-^{14}C -glucose, 1^{14}-C-acetate, H$^{14}CO_3$ and ^{14}C-hexadecane were from the Radiochemical Centre, Amersham.

Radioactive samples were counted on an Intertechnique SL-30 Liquid Scintillation Counter, Nuclear Enterprises, Edinburgh.

Insulin, Oestradiol-3-benzoate, α-Ergocryptine, Epinephrine bitartrate, Bovine Serum Albumin (Fraction V, FFA-free), Palmitic acid and Coomassie Brilliant Blue were from the Sigma Chemical Company.

Hyamine 10x hydroxide, Tannin and Sodium Barbitone were from British Drug Houses.

Intralipid 20% was from Vitrum, Stockholm, Sweden.

Glycerokinase, Pyruvate Kinase, Lactic dehydrogenase, ATP, NADH and Phosphoenol pyruvate were from the Boehringer Corporation.

Bovine prolactin was a gift from the National Institute of Health, Endocrinology Study Section, NIAMDD, Bethesda, U.S.A.

2,5 Diphenyloxazole (PPO) and 1,4-bis(2-(5-phenyloxazoyl)) benzene (POPOP) were from Fisons Scientific Reagents, Loughborough.

Freund's Complete Adjuvant and Incomplete Adjuvant were from Grand Island Biological Co., Grand Island, N.Y.

I.D. Agar Tablets were from Oxoid Ltd., England.

2.2. Methods

2.2.1. Animals

All rats were of the Wistar strain and used in the weight range 200-230g. Mice were of the CD-1 strain and used when about 40g. Animals were either virgin or on their first pregnancy or lactation. They were housed in the animal house, with constant periods of light and dark and fed normal rat fodder.

The day on which sperm was found in vaginal smears from rats or vaginal plugs found in mice was designated day 1 of pregnancy. Pregnancy lasted 22 days in rats and 19 days in mice.

Animals were killed by a blow on the head. Parametrial adipose tissue was used in all experiments unless otherwise stated.

2.2.2. U-^{14}C-Glucose Conversion to $^{14}CO_2$ by Rat Parametrial Adipose Tissue in vitro.

The incubation medium (1 ml) was Krebs-Ringer bicarbonate buffer (106), with half the recommended calcium concentration, D-glucose (10mM; 0.5μCi) and 1 unit of insulin, which produced maximal stimulation of CO_2 release and fatty acid synthesis from U-^{14}C-glucose under these experimental conditions. Insulin was added in 10μl of 0.005M HCl. The gas phase was O_2: CO_2 (95% : 5%) and the pH of the medium was 7.4.

On excision of tissue from the animal, small explants of about 15mg were prepared, gently blotted and weighed. Approximately 50mg of tissue was transferred to each incubation vessel. To obtain a gas-tight seal, the incubations were carried out in glass counting vials fitted with suba-seal stoppers (Figure 3). Small

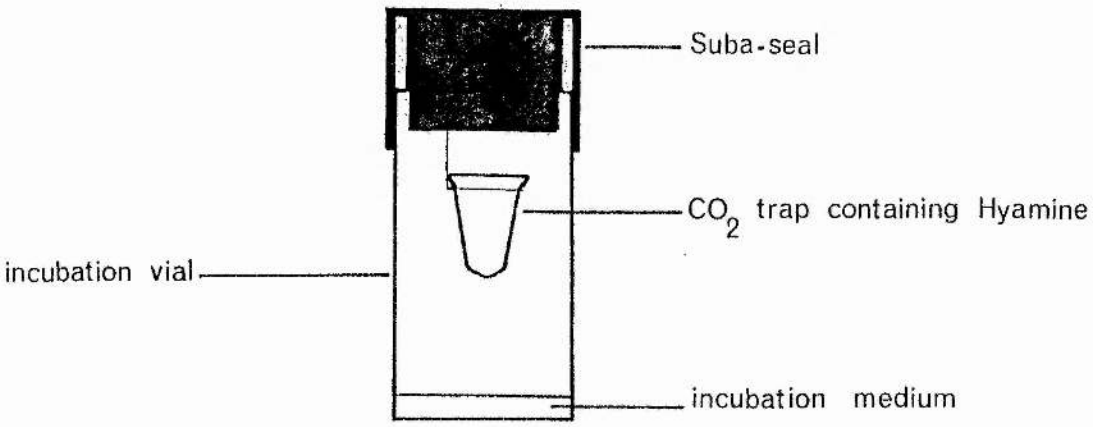


Figure 3. Incubation vessel with CO_2 trap.

glass wells were suspended by wire from the stoppers to hold 0.5ml of Hyamine 10x hydroxide which was used to trap the released CO_2.

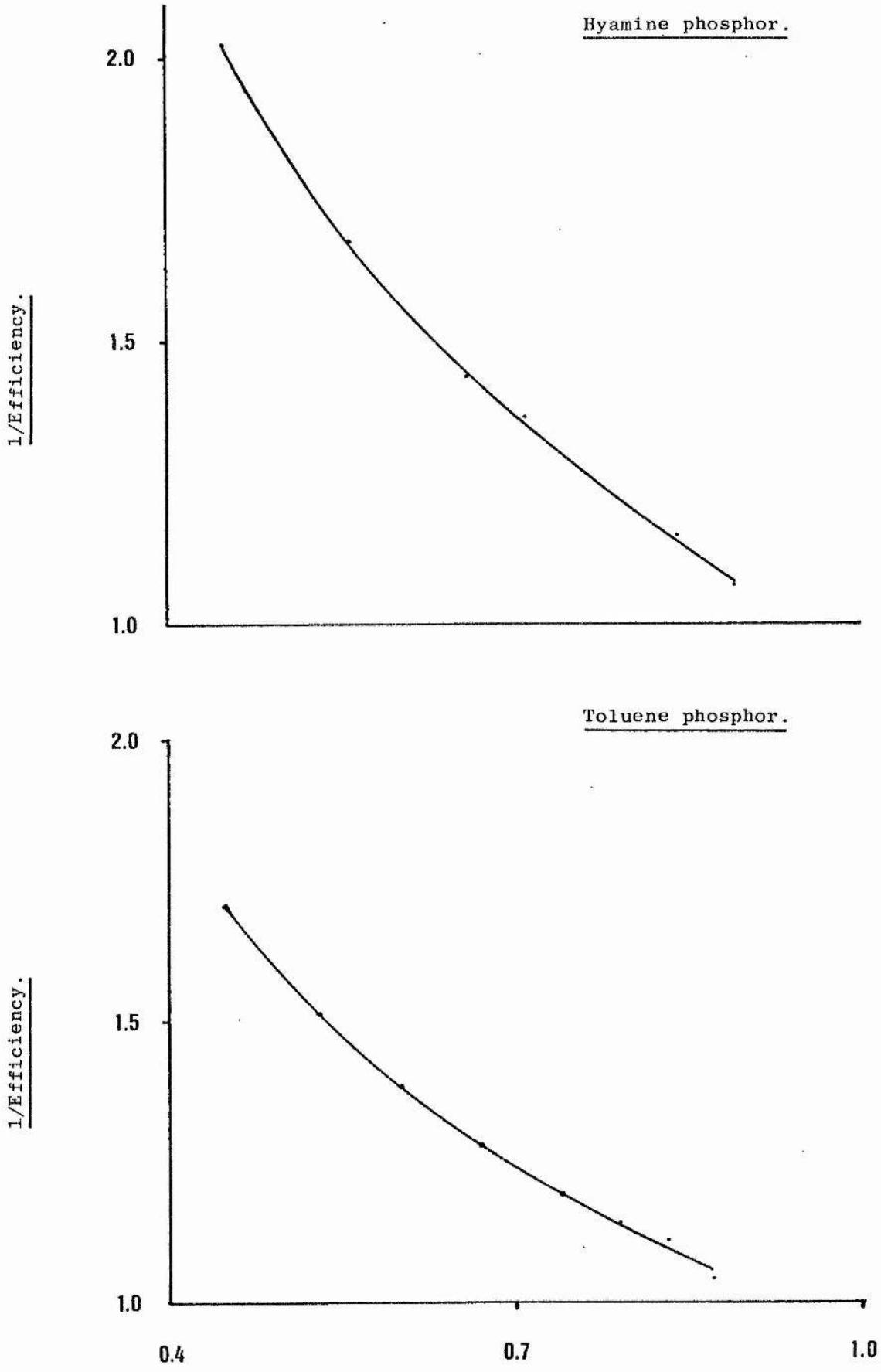
Routinely, incubation time was 180 minutes. Release of CO_2 and synthesis of fatty acids remained linear with time throughout this period and was routinely checked. Incubations were performed on a shaking water-bath maintained at 37°C.

At the end of the incubation period, the reaction was terminated by standing the vials on ice, injecting 0.5ml of Hyamine into the wells, and 0.05ml of concentrated HCl into the incubation medium. Release and trapping of CO_2 was completed on the shaking water-bath at 37°C. The wells were removed from the vials and the outside carefully washed with a water-soaked tissue and wiped dry to remove any labelled glucose which may have splashed onto the well during incubation.

The wells and contents were placed in counting vials containing 12.5ml of liquid scintillator (0.5% PPO, o.o1% POPOP in toluene) and 1.5ml of methanol.

To determine the efficiency of counting of this system, a standard curve was prepared as follows. A series of counting vials were taken containing a known number of disintegrations of ^{14}C-hexadecane in 12.5ml scintillator + 1.5ml methanol. Chloroform was added as quenching agent. The graph shows 1/Efficiency of counting against the channels ratio B : C (Figure 4).

Figure 4. Quench correction curves for liquid scintillation mixtures.



Trapping Efficiency of Hyamine 10x hydroxide

^{14}C-HCO_3 was used to determine the efficiency of trapping. A known amount of ^{14}C-HCO_3 was added to vials containing 1ml KRB buffer, the vials gassed-out with O_2: CO_2 and the suba-seal tops inserted. 0.5ml Hyamine 10x hydroxide was injected into the wells and 0.05ml concentrated HCl into the medium. The vials were placed on a shaking water-bath maintained at 37°C.

Hyamine trapped over 90% of the label from $H^{14}CO_3$ during a 5 minute period and no more CO_2 was trapped after an incubation period of 30 minutes.

Routinely, CO_2 trapping was carried out for 10 minutes at 37°C on a shaking water-bath.

2.2.3. U-^{14}C-Glucose Conversion to Fatty Acids by Rat Adipose in vitro

After the wells had been removed from the vials, the explants and medium were transferred by Pasteur pipette to test tubes. The vials were washed with 1ml of methanol and this was added to the tubes. 0.5ml of aqueous 10N KOH was added and the lipids saponified at 90°C in a water-bath. When saponification was complete, the medium was acidified with concentrated HCl to release free fatty acids, which were extracted twice with 2ml of 40°-60° petroleum ether. A known aliquot was placed in a counting vial and the petroleum ether evaporated under a stream of nitrogen. 5ml of liquid scintillation fluid (0.5% PPO, 0.01% POPOP in toluene) was added. Figure 4 also shows the standard curve for this scintillation mixture.

Lipids for the estimation of tissue lipid content were extracted from adipose tissue by the method of Bligh and Dyer (107).

2.2.4. Estimate of Cell Number of Adipose Tissue

Fat cells were released from adipose tissue lumps by the action of Collagenase Type II, in Krebs-Ringer bicarbonate buffer, plus 4% BSA, in plastic tubes gassed out with $O_2 : CO_2$ (95 : 5). The tubes were incubated on a shaking water-bath for 1 hour. An aliquot of the medium containing released cells was transferred to a microscope slide and cell diameters determined on a calibrated image-splitting microscope. 100 cells from the tissue from each animal were measured. When the cell diameter was known, the mass of one fat cell could be calculated, assuming the density of lipid to be that of triolein (108). The mean fat cell volume was then calculated and the number of cells corresponding to a unit weight of lipid was obtained. All calculations were done by computer.

2.2.5. Lipoprotein Lipase Activity of Adipose Tissue

2.2.5.1. Tissue Preparation and Assay Procedure

About 1g of parametrial adipose tissue was excised from the animal and rinsed in ice-cold saline. The tissue was homogenized in an MSE overhead homogenizer in 70ml cold acetone and filtered through Watman No. 1. Filter paper. The homogenate was washed with 100ml of cold acetone, 200ml acetone at room temperature and 200ml diethyl ether at room temperature. The defatted enzyme preparation was dried overnight at $4^{o}C$ in a vacuum dessicator.

On the day of assay, the dried enzyme preparation was peeled off the filter paper and homogenized gently in ice-cold 25mM

NH_3-HCl buffer, pH 8.1 (5ml buffer per gram wet weight of tissue). After standing 20 minutes on ice, the enzyme solution was centrifuged at 3500 rpm. on a bench centrifuge for 2 minutes. The supernatant was used for the assay of lipoprotein lipase.

Each assay contained:

0.2ml 20% BSA (pH 8.1)

0.1ml 0.7M Tris-HCl buffer (pH 8.1)

0.1ml water

0.05ml rat serum

4% Intralipid emulsion (final concentration).

Serum and Intralipid were pre-incubated together for 30 minutes at 37°C. 0.5ml of enzyme solution was added to start the assay. Routinely, assays were for 1 hour at 37°C on a shaking water-bath.

Figure 5 shows that release of FFA from the emulsion continued in a linear fashion for at least 1 hour.

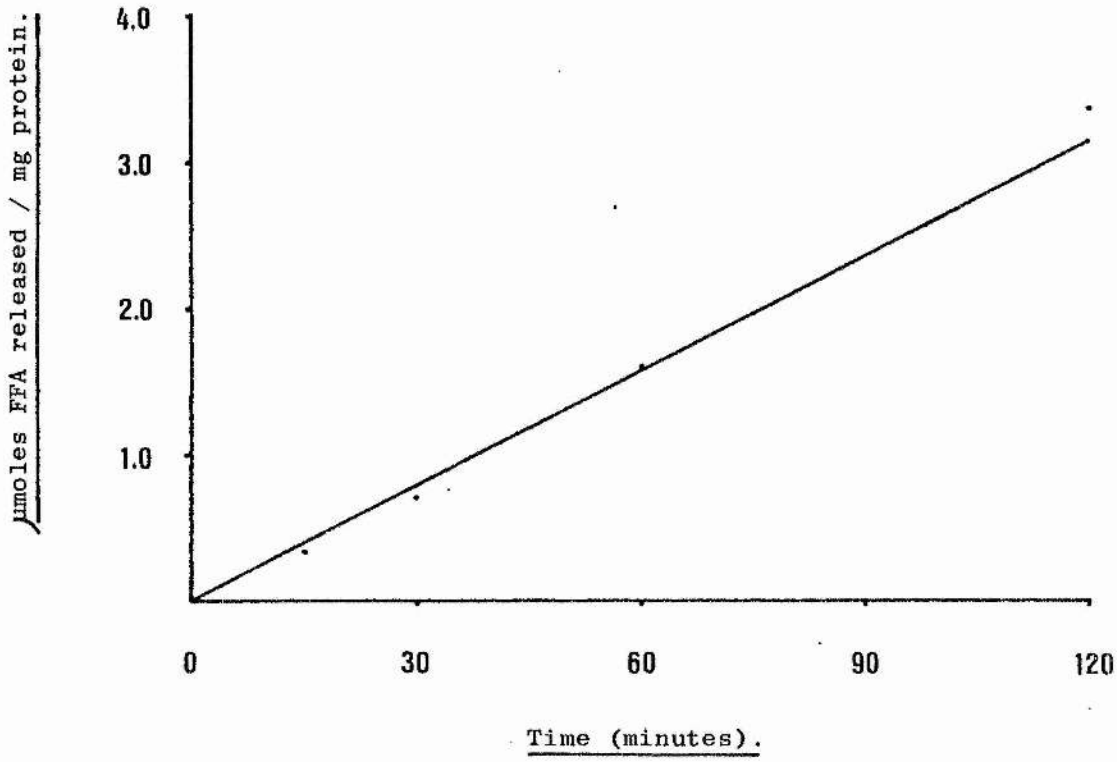
2.2.5.2. Determination of Released Free Fatty Acids (FFA)

Determination of FFA release was carried out essentially by the method of Dole and Meinertz (109). On completion of the incubation period, reaction was terminated by placing the tubes on ice and adding 5.1ml of FFA extraction mixture (Isopropyl alcohol : n-heptane : 1N H_2SO_4 (4:1:0:1)). The tubes were shaken vigorously and allowed to stand for ten minutes. 2ml of water and 3ml of n-heptane were added and the tubes inverted 10 times. On separation of the phases, 2.5 ml of the upper fatty acid-containing heptane layer was dispensed into a test tube and stored at 4°C.

Figure 5. Time course of Release of FFA from the Artificial Emulsion Intralipid by dried, defatted Adipose Tissue Preparation.

Each point is the average of duplicate estimations.

Rat serum was used and tissue was from a virgin rat.



The titration method of Dole (109) was used to determine the amount of FFA in the heptane extract. The indicator, thymol blue, was dissolved in absolute ethanol to a final concentration of 0.01% (w/v). To reduce the titre for the blank, 1N NaOH was added to this solution prior to titration. The solution and gas phase above it were kept saturated with N_2 to prevent CO_2 absorption from the air.

For titration, 2ml of thymol blue indicator was added to tubes containing 2.5ml of fatty-acid-containing heptane. The proportion of solvents, heptane : absolute alcohol of 2.5:2.0 ensured that the solution remained as a single phase throughout titration which facilitated end-point determination. Samples were titrated against approximately 0.018N NaOH, made up with CO_2-free water, and dispensed from a calibrated Agla glass syringe fitted to a microburette. A stream of N_2 was passed through the titration mixture during titration to ensure mixing and prevent CO_2 absorption by the system. A fluorescent lamp was used to illuminate the tubes during titration to facilitate end-point determination. A standard solution of palmitic acid (1.29 μmoles/ 1ml n-heptane) was used daily to standardize the system.

2.2.5.3. Protein Estimation

Protein determination on the lipoprotein lipase extract (see above) was performed (110). 1 volume of LPL extract was mixed with 0.25 volumes of ice-cold 50% TCA. The tube was placed on ice for 1 hour and centrifuged for 10 minutes on a bench centrifuge. The supernatant was decanted and the tube drained.

0.35ml 3% NaOH was added and the tube allowed to stand for 20 minutes at $4^{\circ}C$ to dissolve the pellet. 0.7ml of Biuret

reagent (0.2% AR $CuSO_4-5H_2O$; 0.6% potassium sodium tartrate, in 3% NaOH) was added and the contents mixed. After standing 15 minutes, optical density was determined on a Pye-Unicam SP 500 UV Spectrophotometer at 540nm in Unicam glass cells.

A standard containing 1mg BSA in 3% NaOH was used each day as reference. Figure 6 shows a standard curve for the assay.

2.2.6. Lipolysis in Adipose Tissue.

2.2.6.1. Incubation Procedure

Adipose tissue was quickly excised from the animal and placed in KRB buffer containing 5mM glucose, albumin (40mg/ml) and gassed with $O_2 : CO_2$ (95 : 5). Explants of about 20mg were prepared, gently blotted and weighed. 60-80mg of tissue was transferred to test tubes containing 1ml of medium. Tubes were gassed with $O_2 : CO_2$ (95 : 5) and stoppered. Incubations were carried out for 120 minutes in a shaking water-bath at 37°C. When added, epinephrine bitartrate was present at a concentration of 10µg/ml.

2.2.6.2. FFA Determination

Incubations were terminated by placing the tubes on ice. Explants were removed with forceps to tubes containing 1ml of buffer and 5.1ml of extraction mixture (Isopropanol : n-heptane : 1N H_2SO_4). 5.1ml of extraction mixture was added to tubes containing incubation medium. FFA were determined as described in the previous section.

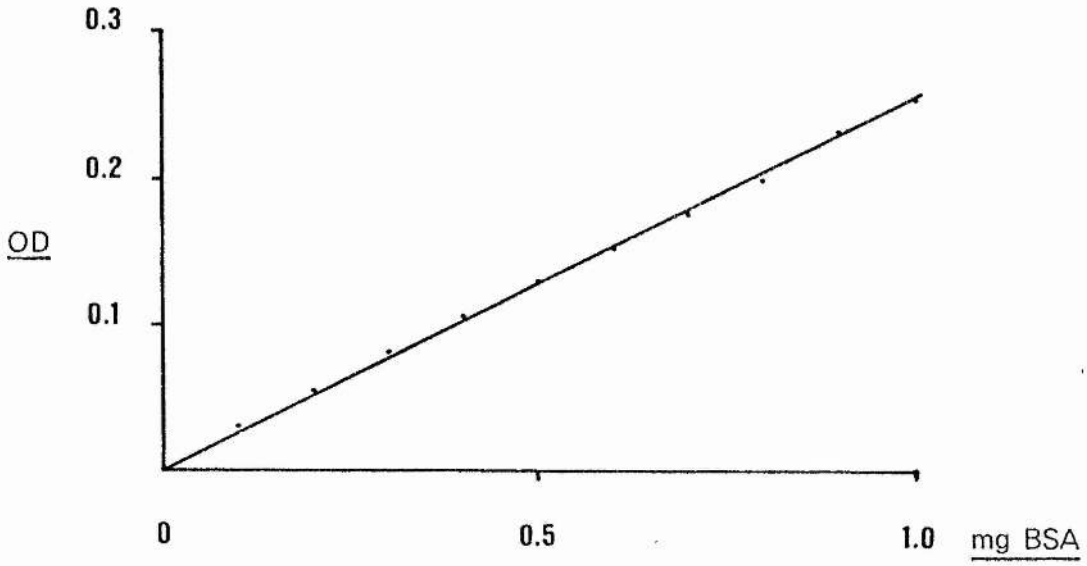


Figure 6. Standard curve for protein estimations.
Experimental conditions were as described in text.
Each point is the average of triplicate determinations.

2.2.6.3. Glycerol Extraction

Incubations (as above) were stopped by placing the tubes on ice and adding 0.05ml of concentrated perchloric acid. About 40mg of $KHCO_3$ was added to neutralize the PCA extract and the tubes centrifuged on a bench centrifuge. An aliquot of the supernatant was retained and frozen prior to glycerol assay.

2.2.6.4. Spectrophotometric Assay of Glycerol

Glycerol was estimated essentially according to the method of Pinter (111). This is a 'linked-assay' system:

$$\text{Glycerol} + \text{ATP} \xrightarrow[\text{Pyruvate kinase}]{\text{Glycerol kinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{ADP} + \text{Phosphoenol-pyruvate} \longrightarrow \text{Pyruvate} + \text{ATP}$$

$$\text{Pyruvate} + \text{NADH} \xrightarrow{\text{Lactate dehydrogenase}} \text{Lactate} + \text{NAD}^+$$

The assay is followed by recording the decrease in absorbance at 340nm due to the conversion of $NADH \longrightarrow NAD^+$.

An assay mixture was made up containing: phosphoenol-pyruvate in triethanolamine buffer (pH 7.0), ATP, NADH (double the recommended concentration), pyruvate kinase and lactic dehydrogenase. The assay of Pinter et al. (111) was modified in that 0.5ml of the reaction mixture was added to 0.5ml of sample solution. 10µl of glycerol kinase solution, containing 4µg of protein, was added to start the reaction after an equilibrium period to obtain a steady baseline. Temperature of the cuvette chamber was maintained at 30°C. Stock glycerol kinase was diluted in triethanolamine buffer and not ammonium sulphate to

prevent spurious absorbance changes as the ammonium sulphate precipitate dissolves in the assay mixture.

A standard glycerol solution (0.2µmol/ml) was used daily as reference. Assays were carried out on a Pye Unicam S.P. 800 UV Spectrophotometer with heated cell carriage and chart recorder.

2.2.7. Production of Prolactin Antiserum

Rabbits of the Dutch strain were used to raise antibodies to prolactin. The injection schedules employed were

a) 1mg prolactin in a water in oil in water emulsion

b) 3mg prolactin in incomplete Freund's adjuvant injected 3 times at 10 day intervals

c) 3mg prolactin in complete Freund's adjuvant injected 3 times at 10 day intervals (112).

All injections were given subcutaneously. A test blood sample was taken via the ear vein 30 days after the first injection.

2.2.8. Tests for the presence of Prolactin Antibodies in Rabbit Serum

The double-diffusion method of Ouchterlony (113) and the tanned-cell technique (114) were used.

2.2.8.1. Ouchterlony Double-Diffusion

Gel was made up as prescribed for Oxoid I.D. Agar Tablets and spread on glass plates to a depth of 1.5mm. Wells to hold 10µl volumes were cut with a gel cutter. Diffusion was carried out at room temperature overnight.

Gels were washed overnight in 0.9% NaCl and in water for 24 hours. This removed unreacted proteins. They were then dried in an oven at 30°C or in a stream of warm air from a hair-drier. Gels were stained with Coomassie Brilliant Blue (1g Coomassie Brilliant Blue : 100ml acetic acid : 450ml ethanol : 450ml distilled water) for 15 minutes and destained for 24 hours with a solution containing 100ml acetic acid, 250ml ethanol, 650ml distilled water (115).

2.2.8.2. Cell-tanning Procedure

Formalinized sheep red-blood cells were prepared and tanned (116). 0.6ml of packed formalinized cells were dispensed into universal bottles, and washed twice with buffered saline (PBS: (pH 7.2); NaCl (0.72%); Na_2HPO_4 (0.148%); KH_2PO_4 (0.043%)). They were suspended in 10ml of this buffer. An equal volume of tannic acid (5mg in 50ml PBS) was added and the bottles incubated for 15 minutes at 37°C. The cells were washed with 20ml of PBS and re-suspended in 10ml of PBS. 10ml PBS containing 2.0mg of prolactin was added and the bottle incubated for 30 minutes at 37°C, to coat the tanned cells with prolactin . The cells were washed 3 times with PBS containing 1% normal rabbit serum (NRS) which was added as a stabilizer. The cells were suspended in this buffer to a concentration of 1%.

Before titration of the antiserum, heterophile agglutins for the red cells were removed. The serum was diluted 1:10 with uncoated tanned cells and left to stand on the bench for 30 minutes. The solution was centrifuged and the supernatant used in the titration.

Titration was carried out in Sterilin plates. 0.1ml of cells (coated or uncoated) was added to 0.1ml of antiserum. Various dilutions of antiserum were employed, details of which are presented in the Results section. Cells were allowed to settle for 24 hours at room temperature.

2.2.9. Effect of Hormones and α-Ergocryptine on the activity of Lipoprotein Lipase in Adipose Tissue in vivo.

Animals used were either virgin, 230g in weight or 1 day lactating. Where lactating animals were used, litters were adjusted to 10 pups per animal and the litter and mother were weighed for 3 days to determine weight changes prior to the injection schedule.

Prolactin was dissolved in a small volume of 0.1M $NaHCO_3$ and made to the required concentration with 0.9% NaCl. Virgin rats received 1mg of prolactin in 0.5ml saline daily as a subcutaneous injection for 7 days.

Oestradiol-3-Benzoate and α-Ergocryptine (for subcutaneous injection) were dissolved in absolute alcohol and emulsified in corn-oil. The ethanol was evaporated under a stream of nitrogen. Animals received daily injections of 0.2ml of oil containing 5μg oestradiol benzoate or 500μg α-ergocryptine, for 7 days. This concentration of oestradiol benzoate has been shown to stimulate prolactin secretion from the pituitary (117) and the α-ergocryptine concentration shown to be capable of blocking oestradiol-stimulated prolactin release (118).

α-Ergocryptine for intraperitoneal injection into lactating rats was prepared as an aqueous emulsion. The ergocryptine was first dissolved in ethanol and then added to 0.9%NaCl to form the emulsion. Animals received 1mg of α-ergocryptine in 0.5ml of saline daily for 3 days. This concentration has been found to block the release of prolactin from the pituitary during lactation in the rat (119).

Removal of tissue from the rats and the assay of lipoprotein lipase were performed as previously described (Section 2.2.5.1. - 2.2.5.3) on completion of the injection schedule.

3. RESULTS

3. RESULTS

3.1.1. Glucose Utilization by Rat Adipose Tissue

Adipose tissue synthesizes fatty acids from glucose and these are mainly esterified with sn-glycerol-3-phosphate to produce storage triglyceride. By following release of $^{14}CO_2$ from ^{14}C-glucose oxidation, glucose utilization can be quantitated and alkaline digestion of adipose tissue with extraction of ^{14}C-fatty acids can be used as an index of the lipid synthetic activity of adipose tissue.

As discussed in the Introduction, lipid substrates may be rerouted to the mammary glands from adipose tissue by reduced synthesis and increased mobilization of triglyceride by adipose tissue in preparation for synthesis of milk fat during lactation. It would be wasteful of energy were the rat to initiate release and continue synthesis of triglyceride in adipose tissue. It is possible, therefore, by following release of $^{14}CO_2$ and synthesis of ^{14}C-fatty acids, to quantitate the changes in glucose utilization by rat adipose tissue during pregnancy and lactation. In this way we may discover if adipose tissue lipid synthesis is reduced during lactation and, if so, when this change occurs. To this end, it was decided to study the utilization of glucose by rat adipose tissue on selected days of pregnancy and lactation.

Much of the previous work done on adipose tissue has involved the epididymal fat pad of the rat. It is known that epididymal adipose tissue responds to the action of insulin and that this response is dose dependent (120). It was decided to use epididymal

adipose tissue in initial experiments to give some indication of the viability of this incubation system. Table 1 shows the results of one such experiment to observe the effect of varying insulin concentrations on the release of CO_2 and synthesis of fatty acids from glucose.

From the work of Cahill (121), a rate of incorporation of 1-^{14}C-glucose into CO_2 of 550nmoles glucose/100mg tissue/3hr can be estimated for epididymal adipose tissue (assuming 2.5mg nitrogen/gram of tissue (122, 123)). Although not identical, comparing the rate with that produced by 1 unit of insulin (Table 1), the results are of a similar order of magnitude. Any discrepancy may be due to the different concentrations of insulin used (Cahill used 10^4μ units of insulin).

Table 1 shows that 1 unit of insulin produced a rate of incorporation of 1-^{14}C-glucose into tissue fatty acid of 637nmoles/100mg tissue/3hr. This is approximately three times the rate reported by Cahill.

100μ units of insulin did not produce an increase in incorporation of 1-^{14}C-glucose into $^{14}CO_2$ by epididymal adipose tissue (Table 1). Increased incorporation of glucose into CO_2 was observed when 500 and 10^6μ units were used.

Incorporation of glucose into adipose tissue fatty acids was decreased on addition of 100μ units of insulin. However, 500 and 10^6μ units of insulin approximately doubled and trebled the rate respectively. The increase in rate produced by 1 unit of insulin was smaller than that shown by Delboca using U-^{14}C-glucose (113).

It was decided to proceed with this incubation system since

Table 1

The Response of Adipose Tissue to Varying Concentrations of Insulin. $^{14}CO_2$ Release and ^{14}C-Fatty Acid Synthesis from 1-^{14}C -Glucose

Insulin (μunits/ml)	nmoles glucose converted/100mg tissue/180 min.	
	$^{14}CO_2$	^{14}C-Fatty Acids
0	588 (±1)	216 (± 20)
100	555 (± 89)	163 (± 16)
500	726 (± 157)	461 (± 51)
10^6	878 (± 155)	637 (± 10)

Krebs-Ringer-bicarbonate buffer (KRB) was the incubation medium. Final concentration of glucose in the medium was 10mM and 0.5μCi of 1-^{14}C -glucose was added to each vessel. Insulin was dissolved in 0.005M HCl and added in 10μl volumes. Each result is the mean of duplicate incubations (± half the range of duplicates).

overall rates were similar to those reported by Cahill using similar concentrations of insulin and 1-^{14}C-glucose and since the tissue responded to different concentrations of insulin. Where added, the insulin concentration in subsequent experiments was 1 unit/ml.

To follow up this experiment, parametrial adipose tissue was used to determine its response to insulin by measuring the conversion of glucose to CO_2 and fatty acids. A time course for this conversion was also studied. The results of this experiment are shown in Table 2.

Doubling the time of incubation resulted in a more than doubling of the conversion of 1-^{14}C-glucose to $^{14}CO_2$ when insulin was present in the medium (Table 2). This is not reflected in the production of ^{14}C-fatty acids from 1-^{14}C-glucose since the rate of conversion was doubled by doubling the incubation time.

Increasing the medium glucose concentration from 10mM to 20mM resulted in a small but insignificant inhibition in the conversion of 1-^{14}C-glucose to $^{14}CO_2$ and ^{14}C-fatty acids.

In the absence of insulin, CO_2 release from glucose by parametrial adipose tissue was less than half that produced in the presence of insulin. This conversion does not show linearity with time. Synthesis of fatty acids from glucose was also much depressed when insulin was omitted from the medium and did not show linearity with time.

Thus, like epididymal adipose tissue, the parametrial fat pad shows sensitivity to insulin in the production of CO_2 and fatty acids from glucose. At 10mM, the glucose concentration is not rate limiting up to 180 minutes since glucose oxidation is

Table 2

The Effect of Insulin on the Conversion of 1-^{14}C -glucose to $^{14}CO_2$ and ^{14}C-Fatty Acid by Rat Parametrial Adipose Tissue and Time Course of this Conversion

Insulin	Glucose (mM)	Time (min)	nmoles glucose incorporated/100mg tissue	
			$^{14}CO_2$	^{14}C-Fatty Acids
+	10	90	293 (± 102)	150 (± 54)
+	10	180	790 (± 70)	299 (± 62)
-	10	90	195 (± 7)	32 (± 2)
-	10	180	320 (± 28)	94 (± 20)
+	20	180	664 (± 38)	269 (± 74)

KRB-buffer, containing 10mM of 20mM and 0.5µCi 1-^{14}C - glucose and insulin at a concentration of 1 unit was the incubation medium. Each result is the mean of duplicate samples (± half the range of duplicates). Tissue was taken from a virgin rat.

approximately linear with time, and increasing the glucose concentration did not result in increased rates.

This sytem has proved to be suitable for routine work with parametrial adipose tissue, the one change in the following experiments being the substitution of U-^{14}C-glucose for 1-^{14}C-glucose. CO_2 release and fatty acid production from glucose was checked routinely for linearity with time. This was maintained throughout the test period.

To determine the glucose utilization and lipid synthesis rate of parametrial adipose tissue during pregnancy and lactation in the rat, conversion of U-^{14}C-glucose to $^{14}CO_2$ and ^{14}C-fatty acids was determined on selected days during the reproductive cycle.

During periods of positive or negative energy balance, a given weight of adipose tissue may contain varying amounts of lipid and cells of different sizes. For this reason, results will be expressed in three ways:

i. nmoles glucose converted/100mg wet weight tissue/180min.

ii. nmoles glucose converted/100mg tissue lipid/180min.

iii. nmoles glucose converted/10^6 fat cells/180min.

In this way, the varying amount of lipid and number of fat cells is allowed for in result expression. This is important, since it has been shown that the capacity of some hormones to cause metabolic changes is different in fat-cells of different sizes (124, 125).

3.1.2. The Effect of Pregnancy and Lactation on the Conversion of U-^{14}C-Glucose to $^{14}CO_2$ by Explants of Rat Parametrial Adipose Tissue.

Table 3 shows the conversion of U-^{14}C-glucose to $^{14}CO_2$ during pregnancy and lactation by rat adipose tissue.

It is clear from the table that the rate of conversion by tissue from virgin animals was much reduced compared with the rates obtained both by epididymal and parametrial adipose tissue using 1-^{14}C-glucose.

The first half of pregnancy is characterized by elevated conversion of glucose to CO_2 by adipose tissue. Tissue from virgin animals converted 150nmoles of glucose/100mg tissue/180min to CO_2 and this was increased by day 5 of pregnancy to 238nmoles of glucose converted. The rate of conversion on day 10 declined to control levels followed by a three-fold rise on day 12. The rate on day 16 was not significantly elevated over controls. By day 19 CO_2 production was less than half of that of virgin animals and continued to decline in early lactation. Tissue from animals on day 8 of lactation showed a small increase in CO_2 production, but this remained lower than virgin control levels.

A similar pattern of conversion of glucose to CO_2 was observed on expressing the results relative to tissue lipid content. The rate of CO_2 production was increased during the first half of pregnancy. A decrease in the rate was again evident on day 10, before a sharp rise by day 12. After day 16 CO_2 production declined until it was less than one third of control levels on day 2 of lactation. The rate increased more than two fold between day 2 and day 8 of lactation.

Table 3. The Effect of Pregnancy and Lactation on the Oxidation of (U-^{14}C)-glucose to $^{14}CO_2$ by explants of Rat Adipose Tissue.

KRB buffer, containing 10 mM and 0.5 μCi (U-^{14}C)-glucose and 1 unit of insulin was the incubation medium. Incubations were carried out and $^{14}CO_2$ was estimated as previously described (2.2.2).

Triplicate samples were performed on tissue from each animal and the mean of these results was calculated. Each result is the mean (± SEM) of the estimations obtained for all animals on each day studied. The number of animals used on each day is shown in parenthesis.

	Day of Pregnancy or Lactation							
	virgin	5	10	12	16	19	2	8
nmoles glucose converted/ 100 mg tissue/180 min.	149.6 ± 19.7	238.8 ± 70.5	148.3 ± 67.3	457.2 ± 285.5	166.3 ± 23.5	68.0 ± 22.8	48.0 ± 6.9	78.8 ± 18.8
nmoles glucose converted/ 100 mg lipid/180 min.	182.5 ± 24.3	288.9 ± 85.5	188.4 ± 85.5	585.8 ± 379.8	213.1 ± 30.3	95.8 ± 32.1	58.0 ± 8.4	124.6 ± 30.1
nmoles glucose converted/ 10^6 cells/180 min.	587.5 + 77.5	1103.6 ± 325.4	725.0 ± 315.6	2132.3 ± 736.0	543.3 ± 76.9	208.9 ± 69.9	181.6 ± 26.0	83.5 ± 19.9
	(3)	(3)	(3)	(6)	(3)	(3)	(3)	(3)

CO_2 production expressed on a fat-cell number basis was similar in pattern to that described above until day 2 of lactation. In contrast to the above results, a further fall in CO_2 production occurred on day 8 of lactation.

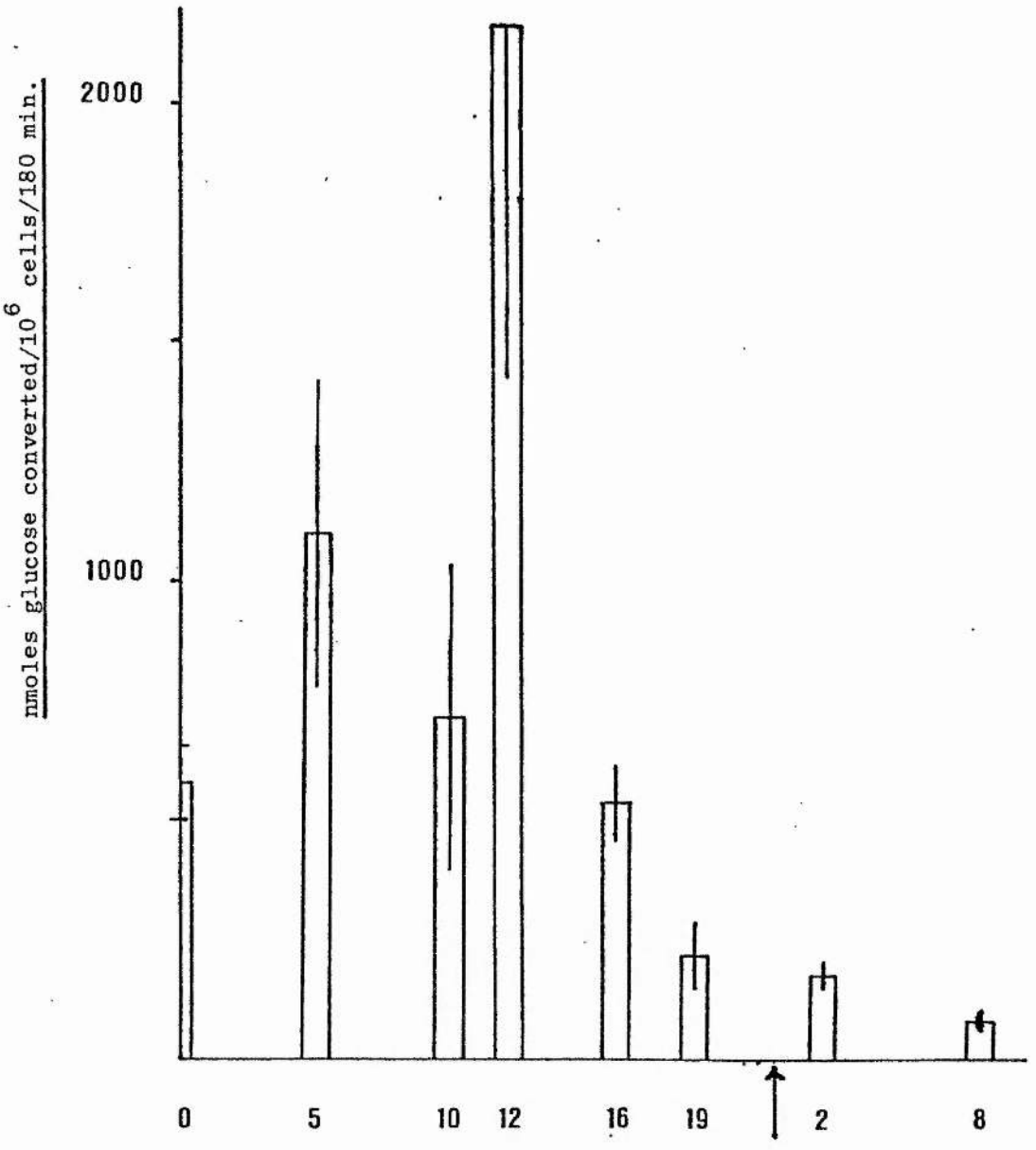
Figure 7 depicts the conversion of glucose to CO_2 expressed as nmoles glucose converted/10^6 fat cells/180min.

It is clear from the above results that utilization of glucose by parametrial adipose tissue in vitro falls into two distinct phases. During the first half of pregnancy glucose oxidation was either elevated or maintained at virgin levels. This was continued until at least day 16 of pregnancy. Thereafter there was a second phase of CO_2 release which was characterized by much reduced rates of production. Results expressed by tissue weight and lipid weight showed a small increase in glucose oxidation between day 2 and 8 of lactation. This suggests that adipose tissue is initiating a recovery of the capacity to oxidize glucose. However, when results are expressed by fat cell number a further fall in rate was evident. This is more consistent with the theory that glucose utilization and lipid synthesis are reduced during lactation in adipose tissue.

It appears, then, that parametrial adipose tissue increases its utilization of glucose during early and mid-pregnancy. In lactation, glucose oxidisation is very much depressed.

Figure 7. The Effect of Pregnancy and Lactation on the Conversion of (U-^{14}C)-Glucose to $^{14}CO_2$ by explants of Rat Adipose Tissue.

Details of experiment were as described in table 3.



*In all figures an arrow represents day of parturition.

3.1.3. The Effect of Pregnancy and Lactation on the Conversion of U-^{14}C-Glucose to ^{14}C-Fatty Acids by Explants of Rat Parametrial Adipose Tissue.

Rates of fatty acid synthesis from glucose expressed on a wet weight tissue basis showed that the conversion of glucose by tissue from animals on days 5 and 10 of pregnancy was not significantly different from that of tissue from virgin animals (Table 4). Fatty acid synthesis was increased four fold in tissue from rats pregnant 12 days. Glucose conversion to fatty acids on day 16 was similar to virgin controls. Adipose tissue from rats on day 19 of pregnancy and days 2 and 8 of lactation showed decreased synthesis of fatty acids from glucose.

Incorporation of glucose into fatty acids expressed on a lipid weight basis did not differ significantly from controls until day 10 of pregnancy. The rate of conversion on day 12 was increased more than four fold compared with day 10 and returned to levels similar to virgin controls on day 16. Fatty acid synthesis from glucose in late pregnancy and lactation was much reduced although a small increase occurred between days 2 and 8 of lactation.

When results are expressed on a fat cell number basis, fatty acid synthesis remains essentially similar throughout pregnancy and early lactation, to that described above. On day 8 of lactation the rate has fallen further and was only approximately 10% of virgin controls.

Figure 8 shows the conversion of U-^{14}C-glucose to ^{14}C-fatty acids by rat adipose tissue expressed on a fat cell number basis.

Thus, there is evidence that the rate of fatty acid synthesis was increased on some days of early- and mid-pregnancy. This was

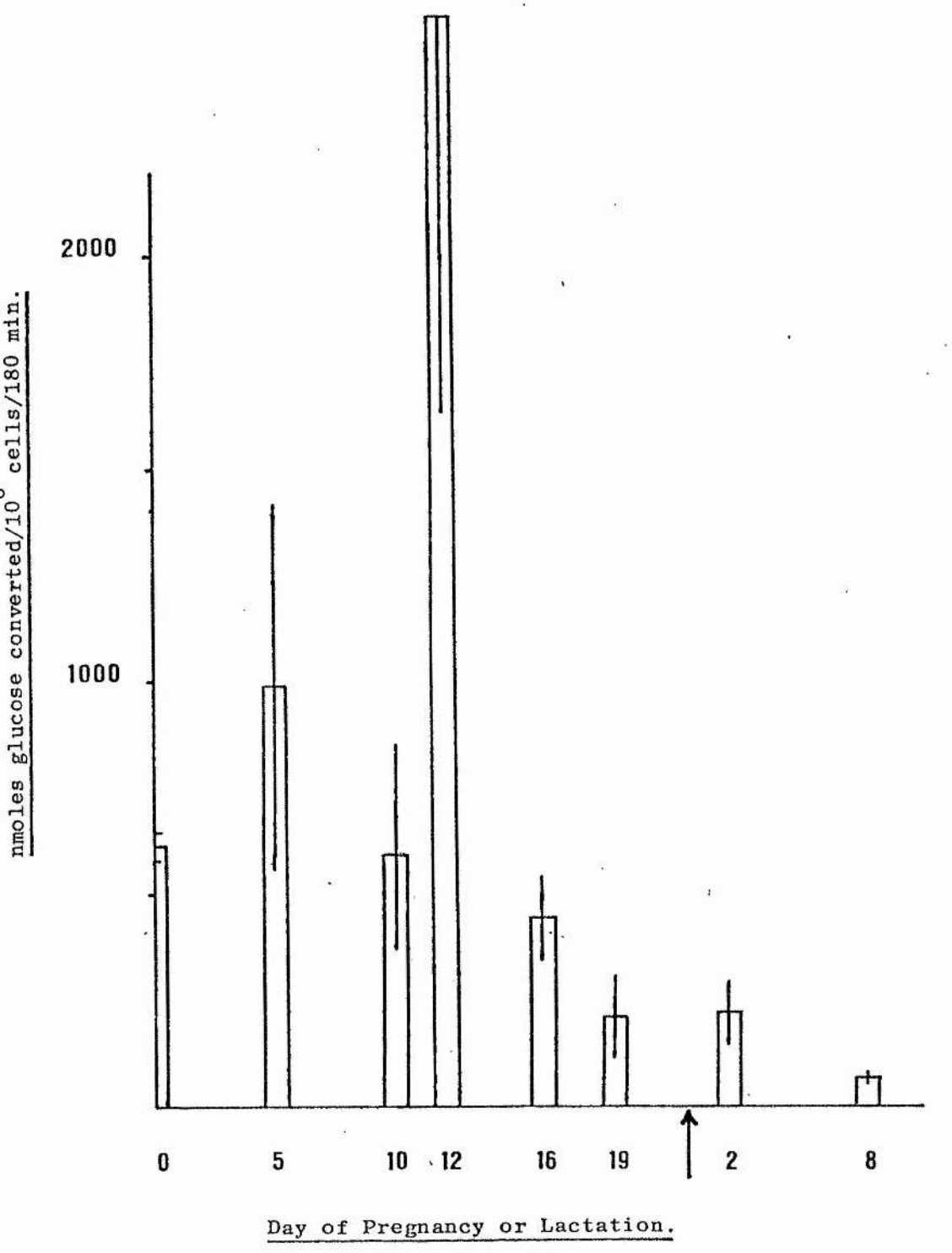
Table 4. The Effect of Pregnancy and Lactation on the Conversion of (U-^{14}C)-glucose to ^{14}C-fatty acids by explants of Rat Adipose Tissue

KRB buffer, containing 10 mM and 0.5 µci (U-^{14}C)-glucose and 1 unit of insulin was the incubation medium. Incubations were carried out and ^{14}C fatty acids estimated as previously described (2.2.3).

Triplicate samples were performed on tissue from each animal and the mean of these results was calculated. Each result is the mean (± SEM) of the estimations obtained for all animals on each day studied. The number of animals used on each day is shown in parenthesis.

	Day of Pregnancy or Lactation							
	virgin	5	10	12	16	19	2	8
nmoles glucose converted/ 100 mg tissue/180 min.	157.1 ± 8.8	215.0 ± 101.2	122.2 ± 51.4	469.4 ± 200.0	138.5 ± 29.5	67.3 ± 31.2	59.3 ± 19.4	59.2 ± 14.7
nmoles glucose converted/ 100 mg lipid/180 min.	191.7 ± 9.8	260.2 ± 121.7	155.9 ± 65.6	703.9 ± 255.1	177.8 ± 38.7	95.0 ± 43.9	71.7 ± 23.6	93.5 ± 22.8
nmoles glucose converted/ 10^6 cells/180 min.	617.3 ± 30.9	994.0 ± 430.6	609.9 ± 241.3	2562.2 ± 928.9	452.0 ± 97.9	207.0 ± 95.7	224.4 ± 73.7	62.7 ± 15.4
	(3)	(3)	(3)	(6)	(3)	(3)	(3)	(3)

Figure 8. The Effect of Pregnancy and Lactation on the Conversion of (U-^{14}C)-Glucose to ^{14}C-Fatty acids by explants of Rat Adipose Tissue.
Details of experiment were as described in table 4.



not true for late pregnancy and lactation when the rate of fatty acid synthesis was consistently low. Comparison of this figure with Figure 7 shows that glucose oxidation and fatty acid synthesis from glucose follow similar patterns. Since glucose oxidation provides acetyl-CoA for fatty acid synthesis, as discussed previously, it would be expected that incorporation of glucose into CO_2 and fatty acids would be similar quantitatively.

One point worth noting is that on day 10 of pregnancy the rate of CO_2 release and fatty acid synthesis returns to virgin control levels whereas on days 5 and 12 increased rates were observed. This will be discussed later.

In summary, parametrial adipose tissue increases utilization of glucose during early- and mid-pregnancy. This may be in preparation for the decreased rate of lipid synthesis during late pregnancy and lactation allowing substrates to be rerouted to the mammary glands for milk synthesis.

3.2. Lipoprotein Lipase Activity of Adipose Tissue

In the previous section, lipid synthesis by parametrial adipose tissue, measured by the incorporation of labelled glucose into fatty acids, was shown to decrease during late pregnancy and lactation. This may allow the mammary glands to utilize more of the available glucose for milk synthesis during lactation. Both adipose tissue and mammary gland utilize blood lipid in the form of chylomicra, whose uptake is regulated by lipoprotein lipase. The most direct way that lipid may be rerouted from adipose tissue to the mammary glands would be decreased uptake by adipose tissue allowing the

blood lipid to be taken up by the mammary glands. Is lipid uptake, as well as lipid synthesis, decreased during late pregnancy and lactation? The activity of lipoprotein lipase in parametrial adipose tissue was therefore studied during pregnancy and lactation.

Histological studies of mouse parametrial adipose tissue revealed what appeared to be a mobilization of storage lipid during late pregnancy and lactation (3). Since this has not been reported for the rat, it was decided that a study of lipid uptake and mobilization in the mouse may provide an interesting comparison with the rat. This section will, therefore, also contain a report on the activity of mouse parametrial adipose tissue lipoprotein lipase.

3.2.1. Requirement of the Assay of Lipoprotein Lipase for Rat Serum as Substrate Activator

The artificial triglyceride emulsion Intralipid was used as substrate in the assay. This has the advantages of stability and economy compared with, for instance, labelled triolein. Chylomicra, the natural substrate, produce an unstable emulsion, as do some of the radioactive emulsions which require daily sonication. For routine work, Intralipid remained stable as an emulsion and the titratable acids in the emulsion did not increase during the period of use.

It was necessary to include serum as substrate activator in the enzyme assay. Table 5 shows the results of an experiment designed to show that the most effective serum in this system was rat serum.

Virgin rat serum produced the highest activity of lipoprotein lipase. Serum from lactating rats and foetal bovine serum did

Table 5

Requirement of the Assay of Lipoprotein Lipase for Rat Serum as Substrate Activator

Serum	Lipoprotein Lipase Activity (μmoles FFA/g)
Virgin rat serum	22.95 ± 0.45
16-day pregnant rat serum	22.80 ± 0
8-day lactating rat serum	20.25 ± 0.15
Foetal bovine serum	13.20 ± 0.30

Tissue was from a virgin rat. Results (± half the range) were the means of duplicate samples. Time of incubation was 60 min. The assay of lipoprotein lipase activity was as previously described (2.2.5.).

not activate the enzyme to the same extent.

It was decided to use serum from fed, virgin rats as substrate activator in all routine assays of lipoprotein lipase activity.

3.2.2. The Effect of Pregnancy and Lactation on the Activity of Rat Parametrial Adipose Tissue Lipoprotein Lipase

Table 6 shows the activity of lipoprotein lipase in rat adipose tissue during pregnancy and lactation. Removal of the acetone-powder enzyme preparation from the surface of the filter paper was not consistent and it came off either in one piece or had to be scraped off almost as a powder. For this reason it was decided to include an expression of results based on the protein recovered. Expression of results by the four methods revealed a similar pattern.

Lipoprotein lipase activity on day 5 of pregnancy fell to approximately one third of virgin controls. An increase in activity occurred through day 10 and by day 12 of pregnancy the activity was almost at control levels. Activity expressed on a protein extract basis on day 12 exceeded that of virgin controls. A small decrease in activity occurred on day 16 except when results were expressed by tissue weight, when activity increased slightly. Enzyme activity on day 19 of pregnancy fell to approximately 25% of virgin controls and to less than 50% expressed on a protein extract basis.

A small increase in activity occurred between day 19 of pregnancy and day 2 of lactation, but by day 8 had fallen to very low levels.

Table 6. The Effect of Pregnancy and Lactation on the Activity of Rat Adipose Tissue Lipoprotein Lipase

The assay of lipoprotein lipase activity was as described previously (2.2.5.1). Triplicate samples were performed on the enzyme extract of tissue from each animal, and the mean of these results was calculated. Each result is the mean (± SEM) of the estimations obtained for all animals on each day studied. The number of animals used on each day is shown in parenthesis.

	Day of Pregnancy or Lactation							
	virgin	5	10	12	16	19	2	8
µmoles FFA/g wet weight/60 min.	29.58 ± 3.18	10.44 ± 2.40	18.00 ± 3.26	22.50 ± 3.94	23.76 ± 2.80	6.33 ± 1.83	8.04 ± 1.71	2.52 ± 0.89
µmoles FFA/100 mg lipid/ 60 min.	3.61 ± 0.39	1.26 ± 0.29	2.22 ± 0.41	3.01 ± 0.54	3.06 ± 0.23	0.85 ± 0.26	0.97 ± 0.22	0.39 ± 0.14
µmoles FFA/10^6 cells/ 60 min.	11.63 ± 1.26	4.81 ± 1.11	8.84 ± 1.63	10.97 ± 1.97	7.77 ± 0.58	1.94 ± 0.59	3.03 ± 0.69	0.26 ± 0.09
µmoles FFA/mg protein/ 60 min.	2.50 ± 0.25	1.33 ± 0.20	2.22 ± 0.25	3.31 ± 0.39	2.83 ± 0.30	1.05 ± 0.20	1.15 ± 0.15	0.50 ± 0.05
	(3)	(3)	(3)	(6)	(3)	(3)	(3)	(3)

Figure 9 and Table 6 show the activity of rat parametrial adipose tissue lipoprotein lipase during pregnancy and lactation.

Except for day 5, the activity of lipoprotein lipase during early and mid-pregnancy was maintained at approximately virgin control levels. Late pregnancy and lactation are characterized by very low levels of activity.

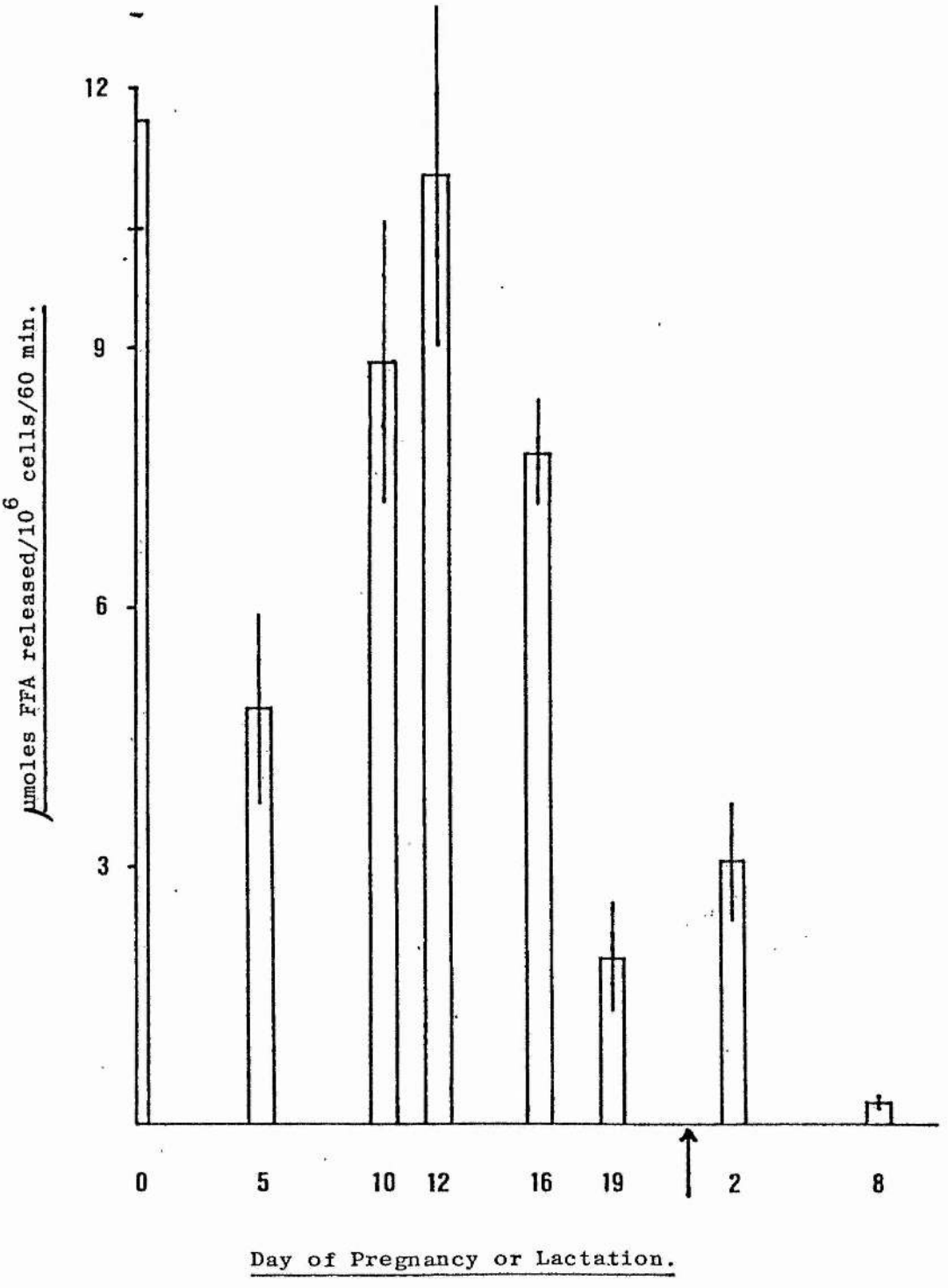
Thus until at least day 16 of pregnancy lipoprotein lipase activity in adipose tissue is more or less maintained at virgin control levels and suggests that the tissue continues to take up and store triglyceride fairly normally until this time. However, consistent with the theory of rerouting of lipid during lactation, enzyme activity during late pregnancy and lactation is very low and lipid uptake is probably negligible.

Comparison of Figure 9 with Figures 7 and 8 reveals that the patterns of glucose utilization, lipid synthesis and lipid uptake are very similar. Adipose tissue increases lipid synthesis and maintains lipid uptake during midgestation. This would have the effect of building up storage lipid in preparation for lactation. Indeed, lipid synthesis and uptake are maintained at low levels during late pregnancy and lactation, thus 'freeing' blood lipid to be used by other tissues, probably mostly by mammary gland.

Since feed intake does not fall during late pregnancy, the drop in lipid synthesis and uptake is not due to lack of substrate - i.e. chylomicra. The factors which cause these changes are most likely hormonal and will be discussed later.

Figure 9. The Effect of Pregnancy and Lactation on the Activity of Rat Adipose Tissue Lipoprotein Lipase.

Details of experiment were as described in table 6.



3.2.3. The Effect of Pregnancy and Lactation on the Activity of Mouse Parametrial Adipose Tissue Lipoprotein Lipase

Table 7 shows the activity of lipoprotein lipase in mouse adipose tissue during pregnancy and lactation.

Enzyme activity fell to approximately 30% of the activity of virgin controls on day 7 of pregnancy. A small rise occurred on days 13 and 15 of pregnancy, but in contrast to rat parametrial lipoprotein lipase, the activity did not rise to control values in mid-pregnancy. The activity increased on day 17 of pregnancy and fell on day 2 of lactation to approximately 50% of virgin controls. This pattern was similar for wet weight, lipid and protein bases. It was hoped to extend these results to include days 18 and 19 of pregnancy and days later in lactation. However not enough parametrial adipose tissue could be obtained from these animals for tests to be completed.

Figure 10 shows the activity of lipoprotein lipase during pregnancy and lactation expressed on a fat cell number basis. Comparison with Figure 9 shows that the activity of lipoprotein lipase in mouse adipose tissue during pregnancy and lactation differs from that of the rat. There is no evidence in the mouse of a build up of lipid stores during early and mid-pregnancy in preparation for mobilization of lipid during lactation. Lipoprotein lipase activity in mouse parametrial adipose tissue is, however, reduced during late pregnancy but not significantly during lactation. Lipid uptake into adipose tissue is thus probably depressed in these animals in late pregnancy compared with virgins but not different during lactation. The total body fat content of the

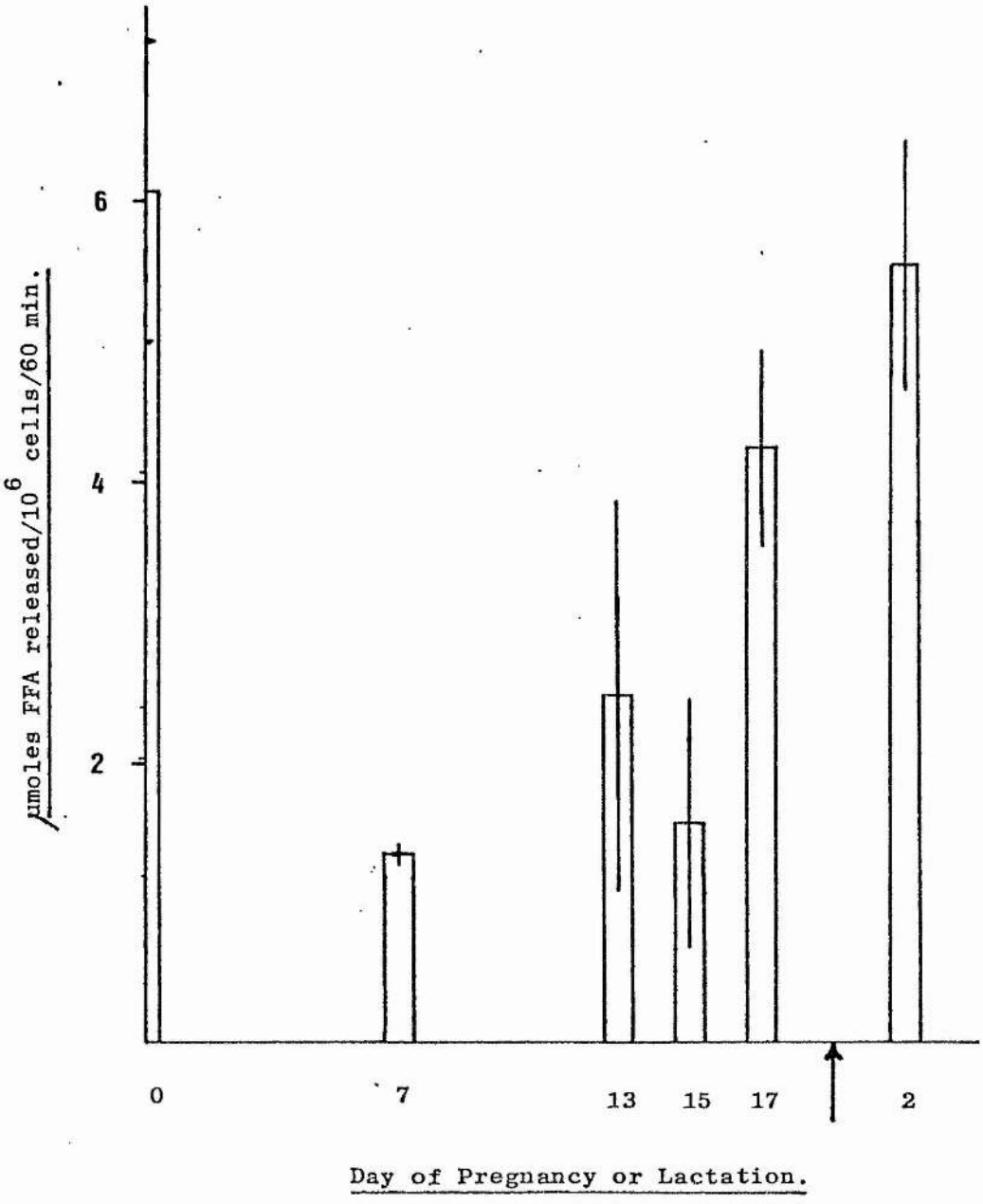
Table 7. The Effect of Pregnancy and Lactation on the Activity of Mouse Adipose Tissue Lipoprotein Lipase

The assay of lipoprotein lipase was as described previously (2.2.5.1). Triplicate samples were performed on the enzyme extract of tissue from each animal, and the mean of these results was calculated. Each result is the mean (± SEM) of the estimations obtained for all animals on each day studied. The number of animals used on each day is shown in parenthesis.

	Day of Pregnancy or Lactation					
	virgin	7	13	15	17	2
µmoles FFA/g wet weight/ 60 min.	25.47 ± 1.59	6.78 ± 0.93	10.68 ± 3.00	8.13 ± 0.81	24.24 ± 1.89	10.23 ± 1.83
µmoles FFA/100 mg lipid/ 60 min.	2.98 ± 0.36	0.86 ± 0.32	1.53 ± 0.85	1.12 ± 0.20	3.67 ± 1.10	1.69 ± 0.48
µmoles FFA/10^6 cells/ 60 min.	6.09 ± 1.07	1.35 ± 0.07	2.48 ± 1.39	1.57 ± 0.90	4.25 ± 0.71	5.57 ± 0.90
µmoles FFA/mg protein/ 60 min.	4.89 ± 0.87	0.63 ± 0.19	1.05 ± 0.19	1.48 ± 0.42	2.63 ± 0.10	1.94 ± 0.29
	(9)	(6)	(9)	(9)	(9)	(9)

Figure 10. The Effect of Pregnancy and Lactation on the Activity of Mouse Adipose Tissue Lipoprotein Lipase.

Details of experiment were as described in table 7.



female mouse increases during pregnancy (5) and this may be due to increased lipid synthesis or uptake by other tissues, since lipid uptake by parametrial adipose tissue is reduced.

Alternatively, the various fat depots may react to pregnancy in different ways resulting in increased lipid deposition into, for example, subcutaneous adipose tissue. Further work is required to clarify this including quantitation of the lipid synthetic rate in mouse adipose tissue during pregnancy and lactation.

3.3. Lipolysis in Parametrial Adipose Tissue

The result of decreased synthesis and uptake of lipid by parametrial adipose tissue during late pregnancy and lactation is increased levels of substrates of triglyceride formation in blood. These substrates are thus made available to the mammary glands for milk synthesis during lactation. However, adipose tissue may contribute to provision of blood lipid for utilization by tissues during the stress of lactation by mobilization of triglyceride. It is likely that some of the fatty acids produced by lipolysis in adipose tissue are incorporated into milk triglyceride by the mammary glands since the majority of fatty acids in adipose tissue are long chain acids, C_{16} and C_{18} (126), and these form the largest fatty acid component in rat milk triglycerides (127).

To determine the extent of adipose tissue lipid mobilization during pregnancy and lactation, lipolysis was quantitated by free fatty acid (FFA) and glycerol release from tissue incubated *in vitro*. It was decided to measure release of both FFA and glycerol since

adipose tissue can reesterify FFA produced by lipolysis with sn-glycerol-3-phosphate produced in glycolysis. Glycerol release is, therefore, the true index of lipolytic rate due to the inability of adipose tissue to re-utilize glycerol to a significant extent (14). The ratio of FFA : glycerol release also gives an indication of the rate of reesterification of FFA in adipose tissue from animals at different stages of the reproductive cycle.

Since a depletion of adipose tissue lipid content had been reported in histological studies during late pregnancy and lactation in the mouse (3), lipolysis was quantitated during pregnancy and early lactation in mouse adipose tissue in an attempt to correlate this with histological evidence, and provide a comparative study with the rat.

Initially, experiments were done using adipose tissue from virgin rats to check the incubation system being used. Release of FFA was first studied under various incubation conditions (Table 8).

Release of FFA from adipose tissue both in the presence and absence of glucose did not exhibit linearity with time up to 120 minutes. When glucose was omitted from the medium, the rate of FFA release in the absence of epinephrine was increased.

Epinephrine-stimulated release of FFA was not linear with time either in the presence or absence of glucose. In the absence of glucose, release of FFA from adipose tissue in response to epinephrine was increased. On average a 5- to 6-fold increase in FFA release was produced by epinephrine stimulation of adipose tissue.

The presence of glucose in the medium restrains the release of FFA from adipose tissue, presumably by reesterification of FFA

Table 8

Release of FFA from Rat Adipose Tissue under Varying Incubation Conditions

Time	Glucose	Epinephrine	µmoles FFA released/100mg lipid
60	+	-	0.56 (± 0.01)
120	+	-	0.65 (± 0.09)
60	+	+	3.10 (± 0.35)
120	+	+	4.43 (± 0.34)
60	-	-	0.69 (± 0.24)
120	-	-	0.94 (± 0.13)
60	-	+	3.58 (± 0.26)
120	-	+	4.83 (± 0.18)

Glucose, where present, was 5mM; albumin was present at 4% (w/v); epinephrine concentration was 10µg/ml, where present.

Incubations were carried out on a shaking water-bath at 37°C. Gas-phase was O_2: CO_2 (95 : 5).

Results are means of duplicate samples (± half the range of duplicates).

with sn-glycerol-3-phosphate. Varying rates of reesterification may account for the failure of the tissue to release FFA linearly with time both with and without epinephrine stimulation. These results confirm those of Lambert (72) both quantitatively and in the general response to varying conditions of incubation.

A further experiment was performed to study the release of both FFA and glycerol from virgin rat adipose tissue *in vitro* (Table 9).

Basal release of FFA from adipose tissue more than doubled when glucose was omitted from the medium. Addition of epinephrine caused a 7-fold increase in FFA release when glucose was present in the medium. Omission of glucose from the medium resulted in increased epinephrine-stimulated FFA release. These results confirm those of the previous experiment regarding the response of adipose tissue to epinephrine stimulation in the absence or presence of glucose. The overall rate of release of FFA is decreased in this experiment which may reflect a different nutritional status of the donor animal.

Basal release of glycerol from adipose tissue in the presence of glucose was approximately linear with time of incubation. Omission of glucose from the medium did not result in altered basal release of glycerol over 120 minutes of incubation.

Release of glycerol from adipose tissue in response to epinephrine stimulation was increased almost 10-fold compared with basal values. Omission of glucose from the medium decreased the epinephrine-stimulated release of glycerol.

Glycerol release in response to epinephrine proceeded approximately linearly with time up to 120 minutes.

Table 9

Release of FFA and Glycerol from Adipose Tissue under Varying Incubation Conditions

Time	Glucose	Epinephrine	μmoles FFA released/100mg lipid
120	+	-	0.34 (± 0.03)
120	-	-	0.88 (± 0.17)
120	+	+	2.40 (± 0.41)
120	-	+	3.20 (± 0.16)
			μmoles glycerol released/100mg lipid
60	+	-	0.07 (± 0.01)
120	+	-	0.11 (± 0.002)
60	+	+	0.47 (± 0.01)
120	+	+	1.08 (± 0.05)
120	-	-	0.10 (± 0.03)
120	-	+	0.68 (0.08)

Glucose concentration was 5mM; albumin was present at 4% (w/v); epinephrine concentration was 10μg/ml.

Incubations were carried out on a shaking water-bath at 37°C. Gas phase was O_2: CO_2 (95 : 5).

Results are means of duplicate samples (± half the range of duplicates).

Basal release of FFA does not proceed linearly with time. This may be due to a relatively larger effect of FFA reesterification on the small rate of FFA release during incubation in the absence of lipolytic stimulation. However, release of glycerol in both the absence and presence of epinephrine proceeded approximately linearly with time. Thus glycerol release will be regarded as the true index of lipolysis in further discussion of results.

It was decided to proceed with tests under the following conditions. (Parametrial adipose tissue was used throughout.) The incubation medium was:

Glucose : 5mM

Bovine serum albumin : 4% (w/v)

Epinephrine (where present) : 10µg/ml.

Krebs-Ringer bicarbonate buffer at half the recommended calcium concentration was used.

The gas phase was O_2: CO_2 (95 : 5). Incubations were carried out on a shaking water-bath at 37°C.

Epinephrine-stimulated glycerol release from adipose tissue is regarded as the true rate of lipolytic response. Basal lipolysis was measured as comparison to epinephrine stimulation, and FFA release measured to indicate the level of reesterification.

3.3.1. Levels of FFA and Glycerol in Rat Adipose Tissue at Zero Time During Pregnancy and Lactation.

During lipolysis, as FFA and glycerol are produced from triglyceride the products accumulate prior to release from the tissue. If this is assumed, when the rate of lipolysis alters, so too should

the levels of FFA and glycerol in the tissue. This may in itself give an indication not only of changes in lipolytic rate, but also of the timing of such changes during pregnancy and lactation.

The levels of FFA and glycerol were therefore estimated in adipose tissue immediately after removal of the tissue from the animal (T_o levels of FFA and glycerol).

Table 10 shows the levels of FFA at T_o in rat adipose tissue during pregnancy and lactation. The pattern of levels of FFA during pregnancy was similar when results were expressed on tissue wet weight, lipid weight and fat cell number bases. No significant change occurred until day 16 of pregnancy. However, the levels were increased on day 19. Further small increases were evident when results were expressed on wet weight and lipid bases during lactation. On a cell number basis the levels of FFA on day 3 of lactation were increased more than four fold compared with controls. The levels on day 10 were lower than controls, presumably due to the increased number of cells per unit weight of tissue found at this time.

If these results are compared with those for glycerol levels (Table 11), it is clear that the patterns are similar. No significant increases occurred during pregnancy although increases in mean levels were evident on days 7 and 16. In contrast to FFA levels, no increase in glycerol levels was seen in tissue from 19-day pregnant animals. However, on all result expression methods, glycerol levels were increased during early lactation. By day 10 of lactation T_o Glycerol levels had decreased.

It appears that rat adipose tissue does not show evidence of increased levels of FFA until late pregnancy and glycerol levels

Table 10. Levels of FFA in Rat Adipose Tissue at Zero Time during Pregnancy and Lactation.

FFA were estimated as previously described (2.2.5.2). Duplicate estimations were performed on tissue from each animal and the mean calculated. Each result is the mean (± SEM) of all estimations on tissue from all animals on each day studied. The number of animals on each day was 3.

	Day of Pregnancy or Lactation						
	virgin	7	12	16	19	3	10
µmoles FFA/100 mg tissue	0.39 ± 0.04	0.34 ± 0.04	0.31 ± 0.04	0.37 ± 0.02	0.60 ± 0.04	0.79 ± 0.15	0.86 ± 0.19
µmoles FFA/100 mg lipid	0.48 ± 0.07	0.41 ± 0.04	0.43 ± 0.04	0.48 ± 0.05	0.85 ± 0.06	0.96 ± 0.20	1.35 ± 0.35
µmoles FFA/10^6 cells	1.58 ± 0.28	1.56 ± 0.49	1.60 ± 0.72	1.22 ± 0.11	1.86 ± 0.14	7.56 ± 3.60	0.91 ± 0.23

Table 11. Levels of Glycerol in Rat Adipose Tissue at Zero Time during Pregnancy and Lactation

Glycerol was estimated as previously described (2.2.6.3).

Conditions and estimations were as described in table 10.

	Day of Pregnancy or Lactation						
	virgin	7	12	16	19	3	10
µmoles glycerol/100 mg tissue	0.04 ± 0.01	0.06 ± 0.03	0.04 ± 0.01	0.07 ± 0.02	0.03 ± 0.01	0.16 ± 0.06	0.08 ± 0.01
µmoles glycerol/100 mg lipid	0.04 ± 0.00	0.07 ± 0.03	0.05 ± 0.01	0.09 ± 0.02	0.04 ± 0.01	0.21 ± 0.09	0.13 ± 0.02
µmoles glycerol/10^6 cells	0.13 ± 0.04	0.27 ± 0.15	0.18 ± 0.01	0.23 ± 0.05	0.08 ± 0.01	1.38 ± 0.22	0.09 ± 0.01

until early lactation. If this is assumed to reflect increased lipolytic rate, the stimulus to increase lipolysis appears to be present in early lactation. The increase in tissue levels was not, however, maintained into later lactation.

3.3.2. The Effect of Pregnancy and Lactation on FFA Release by Rat Parametrial Adipose Tissue in vitro

Table 12 shows the rate of basal lipolysis measured by FFA release by adipose tissue in vitro from rats on various days of pregnancy and lactation.

Expression of results on a tissue wet weight basis shows that basal lipolytic rate increased on days 7 and 12 of pregnancy compared with virgin control level. The rate then decreased, returning to levels similar to controls by day 19 of pregnancy. The rate of basal lipolysis increased on days 3 and 10 of lactation.

Results expressed on a tissue lipid basis show that the mean basal lipolytic rate was increased, but not significantly, on days 7, 12, 16 and 19 of pregnancy. The rate on day 3 postpartum was not significantly different from virgin control levels. A two fold increase in basal lipolysis had occurred by day 10 of lactation.

Figure 11 shows the effect of pregnancy and lactation on basal lipolysis in rat adipose tissue in vitro measured on a fat cell number basis. The pattern of basal lipolysis was similar to that when results were expressed on a tissue wet weight basis. The mean rate was increased on days 7 and 12 of pregnancy and thereafter declined until it was lower than controls on day 19. Release of FFA almost trebled on day 3 of lactation. However, by day 10 release was decreased, although not significantly, compared with controls.

Table 12. The Effect of Pregnancy and Lactation on FFA Release by Rat Adipose Tissue 'in vitro'.

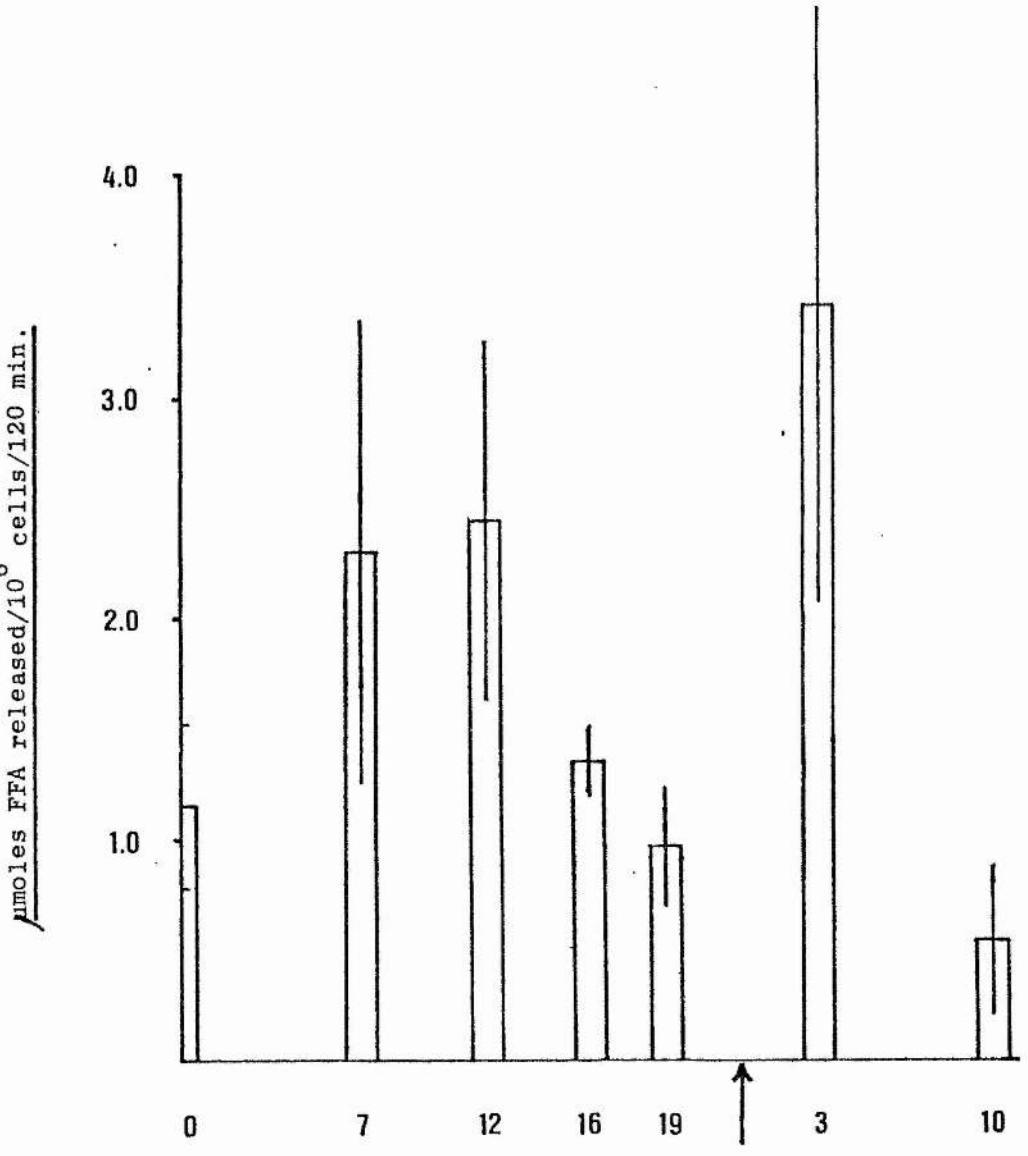
KRB buffer containing 5 mM glucose and 4% albumin was the incubation medium. Time of incubations was 120 minutes and the gas phase was O_2:CO_2(95: 5%).

Triplicate samples were performed on tissue from each animal and the mean of these results was calculated. Each result is the mean (± SEM) of all estimations on tissue from all animals on each day studied. The number of animals used on each day was 3.

	Day of Pregnancy or Lactation						
	virgin	7	12	16	19	3	10
µmoles FFA/100 mg wet weight/120 min.	0.30 ± 0.10	0.49 ± 0.19	0.48 ± 0.13	0.41 ± 0.03	0.30 ± 0.10	0.39 ± 0.16	0.51 ± 0.25
µmoles FFA/100 mg lipid/120 min.	0.37 ± 0.13	0.60 ± 0.28	0.66 ± 0.22	0.52 ± 0.06	0.43 ± 0.15	0.47 ± 0.21	0.80 ± 0.45
µmoles FFA/10^6 cells/ 120 min.	1.15 ± 0.37	2.32 ± 1.05	2.46 ± 0.82	1.35 ± 0.16	0.97 ± 0.27	3.45 ± 1.36	0.54 ± 0.35

Figure 11. The Effect of Pregnancy and Lactation on FFA Release by explants of Rat Adipose Tissue.

Details of experiment were as described in table 10.



In general, basal lipolysis measured by FFA release was insignificantly elevated during early- to mid-pregnancy. Late pregnancy was characterized by very low levels of basal lipolysis and when results were expressed on a lipid weight and wet weight bases there was indication of increased lipolysis during later lactation.

If the rate of release of FFA from adipose tissue incubated *in vitro* is assumed to be a reflection of the conditions *in vivo*, there is evidence for increased lipid mobilization during lactation in the rat. There is, however, evidence to suggest that FFA release may be elevated during early- and mid-pregnancy. This is surprising, since FFA synthesis is elevated at this time.

Increased sensitivity of adipose tissue to the action of epinephrine indicates that the tissue is increasingly responsive to a lipolytic stimulant. By exposing tissue to maximal concentrations of this hormone it was hoped to discover if sensitivity alters during pregnancy and lactation thus providing further information on lipid mobilization.

3.3.3. The Effect of Pregnancy and Lactation on the Response of Rat Adipose Tissue *in vitro* to Epinephrine Stimulation.

Release of FFA.

The rate of epinephrine-stimulated lipolysis, measured by FFA release, during pregnancy and lactation is shown in Table 13 and Figure 12. The pattern during pregnancy was similar when results were expressed by the three different methods. Increased mean rates, compared with virgin controls, were evident on days 7, 12, 16 and 19 of pregnancy.

Table 13. The Effect of Pregnancy and Lactation on the Response of Rat Adipose Tissue to Epinephrine Stimulation 'in vitro'. Release of FFA.

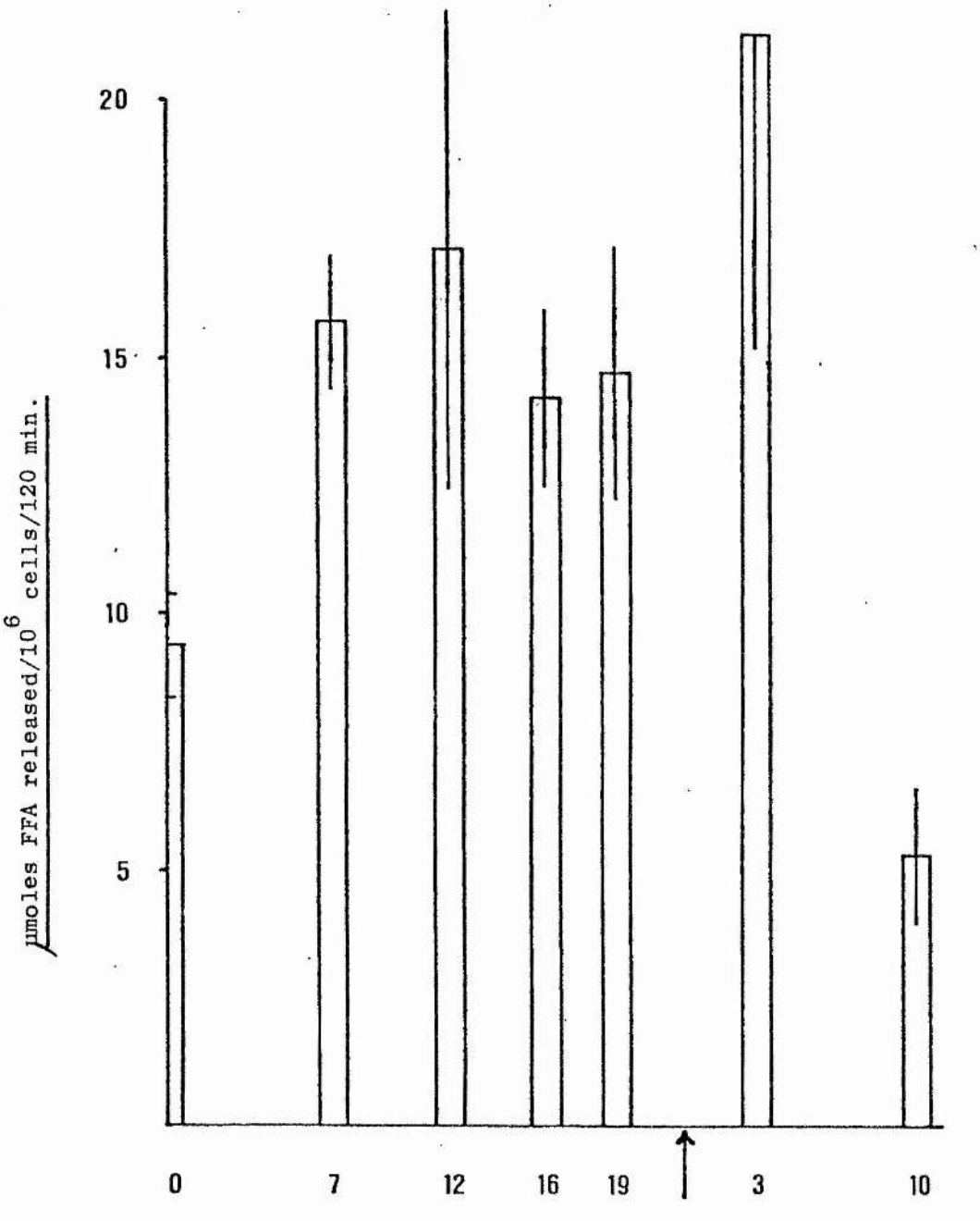
KRB buffer, containing 5 mM glucose, 4% albumin and 10 µg/ml of epinephrine was the incubation medium. Incubations were performed for 120 minutes and the gas phase was O_2: CO_2 (95 : 5%).

Triplicate samples were performed on tissue from each animal and the mean of these results was calculated. Each result is the mean (± SEM) of the estimations obtained for all animals on each day studied. The number of animals used on each day was 3.

	Day of Pregnancy or Lactation						
	virgin	7	12	16	19	3	10
µmoles FFA/100 mg tissue/ 120 min.	2.37 ± 0.19	3.26 ± 0.36	3.30 ± 1.04	4.42 ± 0.83	4.59 ± 0.29	2.41 ± 0.37	4.85 ± 1.27
µmoles FFA/100 mg lipid/ 120 min.	2.91 ± 0.29	3.95 ± 0.53	4.52 ± 1.14	5.57 ± 0.51	6.48 ± 0.26	2.89 ± 0.83	7.68 ± 1.91
µmoles FFA/10^6 cells/ 120 min.	9.37 ± 1.00	15.71 ± 1.29	17.00 ± 4.63	14.18 ± 1.71	14.67 ± 2.46	21.19 ± 6.08	5.15 ± 1.34

Figure 12. The Effect of Pregnancy and Lactation on the Response of Rat Adipose Tissue 'in vitro' to Epinephrine Stimulation. Release of FFA.

Details of experiment were as described in table 11.



Epinephrine stimulated lipolysis, when results are expressed on tissue wet weight and tissue lipid bases, returned to control levels on day 3 of lactation. By day 10 postpartum, the rate was increased compared with virgin controls.

In contrast, the rate expressed by fat cell number more than doubled on day three of lactation and then decreased by day ten postpartum to a rate lower than that of virgin controls. On day 10, the fat cell number per unit weight of tissue had increased. This may be due to depletion of the fat cells of lipid if lipolysis is increased during lactation. The increased cell count would also depress the mean rate on day 10 postpartum.

The response of adipose tissue to stimulation by epinephrine is increased throughout pregnancy, the timing of this increased sensitivity being as early as day 7. This is perhaps surprising at a time when milk fat synthesis has not been initiated and lipid synthesis in adipose tissue is increased (Table 8). It may be that lipolysis is initiated early in pregnancy and the FFA so produced used as a fuel source by the animal. Alternatively, adipose tissue may somehow be sensitized during early pregnancy to respond to a lipolytic stimulus during pregnancy and/or lactation.

Since it has been reported that the mass of rat parametrial adipose tissue does not alter significantly during pregnancy (57), it seems unlikely that net lipolysis is increased <u>in vivo</u>. However, it is reported here and elsewhere (56, 57) that lipoprotein lipase maintains or increases activity during mid-pregnancy, and this, with increased FFA synthesis (Table 8), may compensate for increased lipolysis.

3.3.4. The Effect of Pregnancy and Lactation on Glycerol Release by Rat Parametrial Adipose Tissue in vitro

Basal lipolysis in rat adipose tissue during pregnancy and lactation, measured by glycerol release, is shown in Table 14.

No significant difference in glycerol release occurred on days 7, 12 and 19 of pregnancy when results were expressed on a tissue wet weight basis. Tissue from animals on day 16 of pregnancy and days 3 and 10 of lactation showed elevated levels of basal lipolysis.

Comparison of these results with those for basal FFA release (Table 12) shows that the results do not follow the same pattern with the exception of days 16 and 19 of pregnancy. Surprisingly, FFA release is increased on days 7 and 12 of pregnancy whereas glycerol release does not vary from virgin controls. In contrast, glycerol release is increased during lactation and FFA release is not. The latter may be explained by increased rates of reesterification of FFA.

When results were expressed on a lipid basis, two phases of glycerol release were evident. During early pregnancy glycerol release was not significantly different from controls. Thereafter, the rate of release increased on days 16 and 19 of pregnancy and continued until day 10 postpartum. If this is compared with Table 12 it can be seen that FFA release is elevated throughout pregnancy and lactation and correlates with the results presented here for basal glycerol release.

Figure 13 shows the rate of basal lipolysis in rat adipose tissue *in vitro* during pregnancy and lactation expressed on a

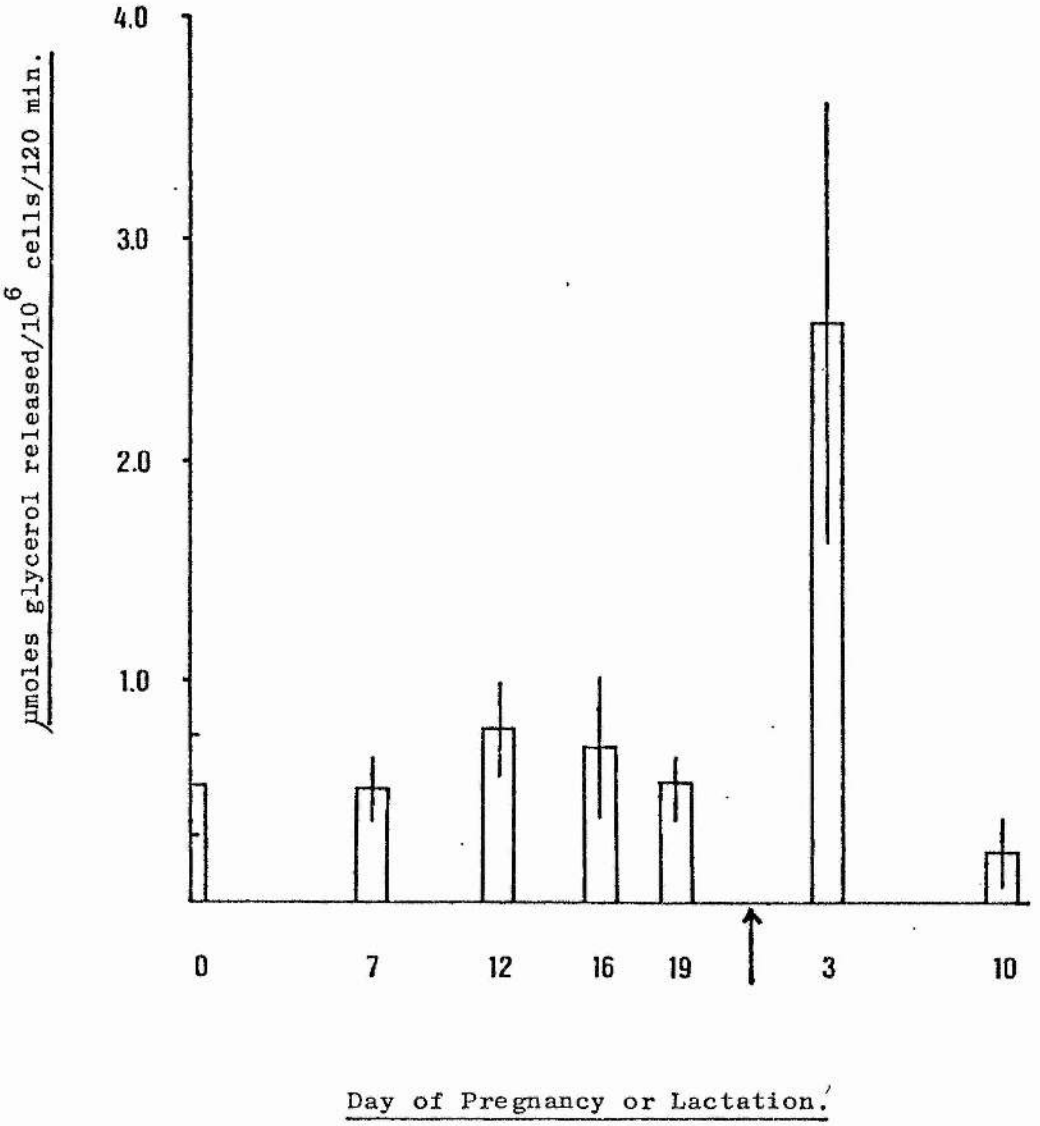
Table 14. The Effect of Pregnancy and Lactation on Glycerol Release by Rat Adipose Tissue 'in vitro'.

Conditions of incubations and expression of results are as described in table 12.

	Day of Pregnancy or Lactation						
	virgin	7	12	16	19	3	10
µmoles glycerol/100 mg tissue /120 min	0.13 ± 0.01	0.11 ± 0.04	0.14 ± 0.04	0.23 ± 0.10	0.17 ± 0.03	0.30 ± 0.04	0.22 ± 0.07
µmoles glycerol/100 mg lipid/ 120 min.	0.17 ± 0.01	0.13 ± 0.06	0.21 ± 0.06	0.29 ± 0.11	0.24 ± 0.08	0.36 ± 0.13	0.35 ± 0.15
µmoles glycerol/10^6 cells/ 120 min.	0.53 ± 0.23	0.50 ± 0.15	0.79 ± 0.21	0.74 ± 0.32	0.55 ± 0.12	2.64 ± 1.00	0.23 ± 0.16

Figure 13. The Effect of Pregnancy and Lactation on Glycerol Release by explants of Rat Adipose Tissue.

Details of experiment were as described in table 12.



fat-cell number basis. Release of glycerol was increased over virgin control levels only on day 3 of lactation. Comparison of Figure 11 with Figure 13 reveals that basal lipolysis measured by FFA and glycerol release is essentially similar. No definite trend in basal lipolysis is evident with rates mostly similar to virgin controls. However, increased FFA and glycerol release during early lactation provides evidence that lipid may be mobilized from adipose tissue in vivo at this time.

The results confirm the benefits of expressing results on a lipid or fat cell number basis, since only when these were employed did lipolysis measured by FFA and glycerol release show correlation.

3.3.5. The Effect of Pregnancy and Lactation on the Response of Rat Adipose Tissue in vitro to Epinephrine Stimulation. Release of Glycerol.

The sensitivity of adipose tissue to a lipolytic stimulus was also studied by incubating the tissue with maximal concentrations (i.e. 10µg/ml) of epinephrine.

Table 15 shows the results of experiments designed to quantitate the response of rat adipose tissue in vitro to epinephrine stimulation during pregnancy and lactation.

Release of glycerol, expressed by tissue wet weight, was substantially different from virgin controls on only day 16 and 19 of pregnancy when the rate was increased.

Expressing results on a tissue lipid basis showed that epinephrine-stimulated lipolysis was increased on days 16 and 19 of pregnancy but was not significantly different from virgin controls on the other days studied.

Table 15. The Effect of Pregnancy and Lactation on the Response of Rat Adipose Tissue to Epinephrine Stimulation 'in vitro'. Release of Glycerol.

Conditions of incubations and expression of results are as described in table 13.

	Day of Pregnancy or Lactation						
	virgin	7	12	16	19	3	10
µmoles glycerol/100 mg tissue/ 120 min.	1.24 ± 0.09	1.08 ± 0.22	1.35 ± 0.44	2.11 ± 0.74	2.09 ± 0.34	0.98 ± 0.19	1.20 ± 0.14
µmoles glycerol/100 mg lipid/ 120 min.	1.53 ± 0.19	1.31 ± 0.29	1.83 ± 0.55	2.63 ± 0.86	2.95 ± 0.36	1.18 ± 0.30	1.92 ± 0.23
µmoles glycerol/10^6 cells/ 120 min.	4.91 ± 0.19	5.07 ± 0.44	6.88 ± 1.14	6.84 ± 2.59	6.57 ± 0.82	8.92 ± 1.66	1.31 ± 0.36

Epinephrine-stimulated lipolysis expressed by fat cell number is depicted in Figure 14. The response of rat adipose tissue to epinephrine stimulation was increased from day 12 of pregnancy until day 3 of lactation. By day 10 postpartum the lipolytic rate had fallen to approximately 30% of virgin control levels. As previously discussed, this may be due to increased fat cell count per unit weight of tissue resulting from lipid depletion.

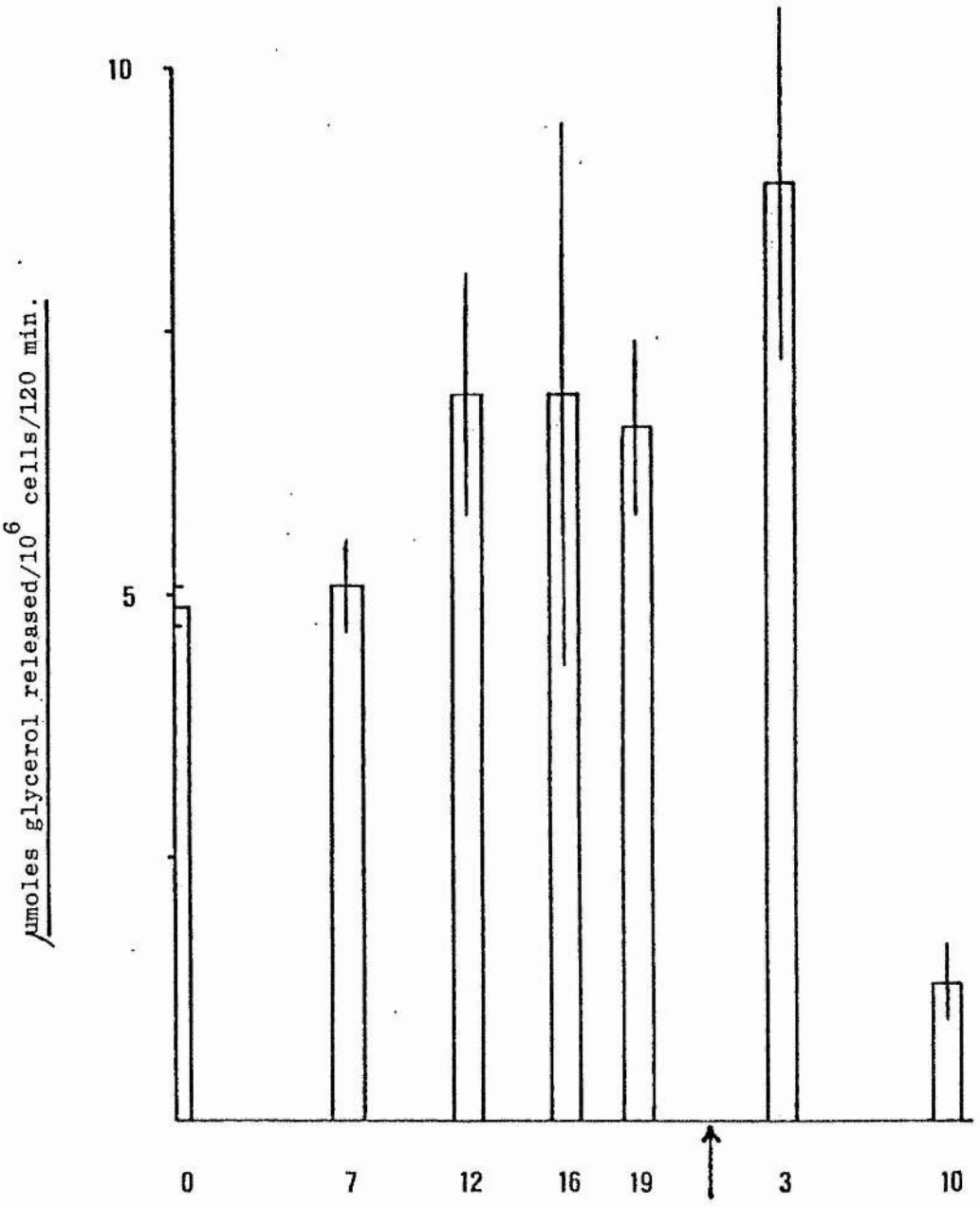
Comparison of Table 15 with Table 13 shows that when results are expressed on tissue wet weight and tissue lipid bases, there is poor correlation between FFA release and glycerol release. However, if Figures 12 and 14 are compared, it can be seen that when fat cell number is used to express results, correlations between FFA and glycerol release is good.

The results presented for glycerol release confirm those presented earlier (Fig. 12) that the response of adipose tissue to the action of epinephrine is increased during pregnancy and that this increase is continued into early lactation. No evidence was found for increased sensitivity later in lactation.

In studying lipolysis by release of FFA from adipose tissue, the FFA contents of the tissue and medium following incubation were measured separately and combined to give total FFA produced via lipolysis. However, the distribution of FFA in tissue and medium following incubation may provide information on the rate of re-esterification of FFA by adipose tissue during pregnancy and lactation.

Figure 14. The Effect of Pregnancy and Lactation on the Response of Rat Adipose Tissue 'in vitro' to Epinephrine Stimulation. Release of Glycerol.

Details of experiment were as described in table 13.



3.3.6. The Changes in FFA Accumulation by Rat Adipose Tissue in vitro during incubation to study Basal Lipolysis during Pregnancy and Lactation

Figure 15 shows the changes in adipose tissue FFA content during pregnancy and lactation. Results are presented as net percent loss or gain of FFA by adipose tissue relative to the FFA content at the beginning of incubation. Negative results indicate loss of FFA either to the medium or in reesterification.

It is clear that, on all days studied except days 12 and 16 of pregnancy, following incubation without lipolytic stimulation, the FFA content of adipose tissue decreased. This was most evident during late pregnancy and lactation. FFA content of adipose tissue on days 12 and 16 of pregnancy had increased following a two hour incubation period.

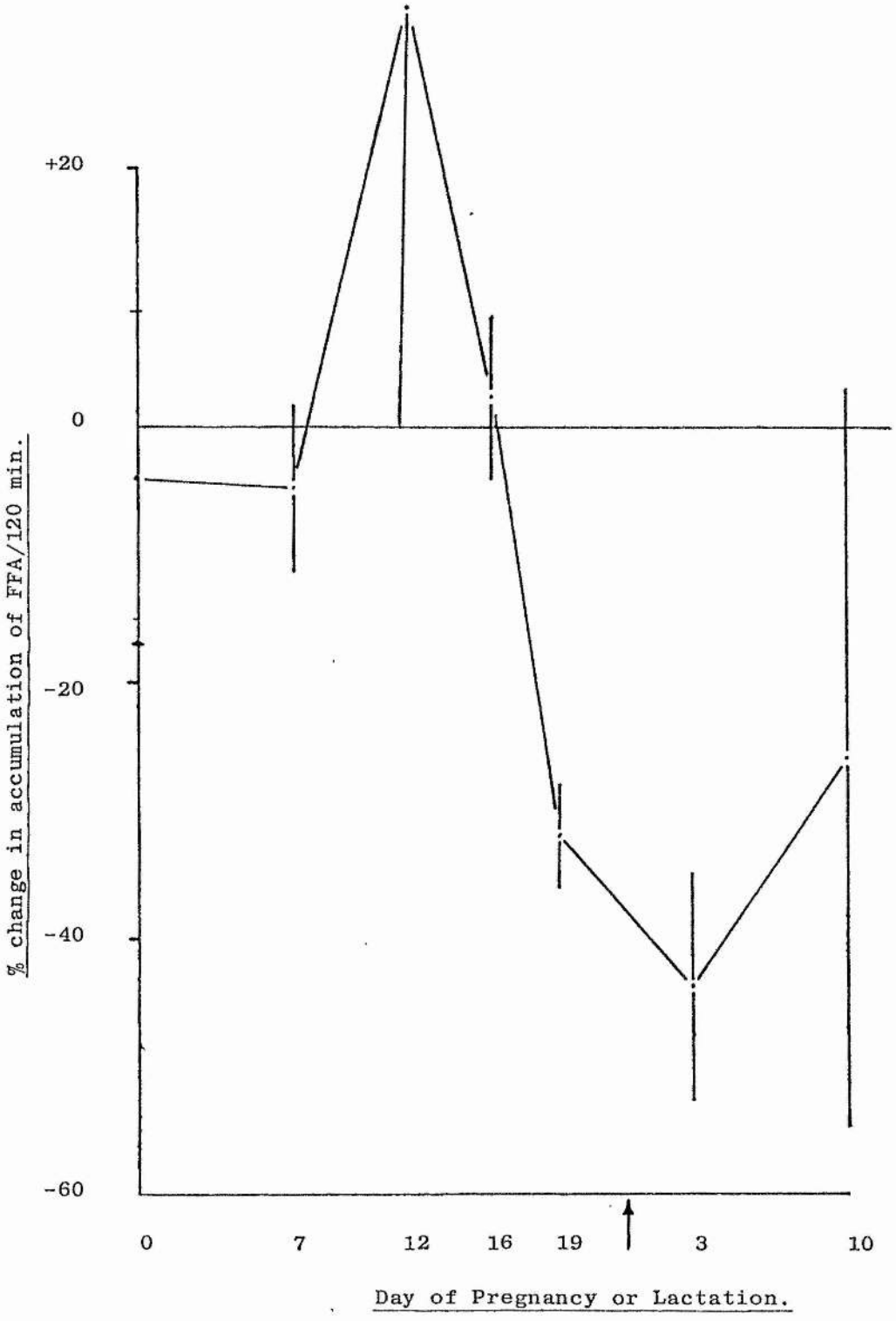
Comparison of Figures 15 and 11 shows that when the content of FFA in adipose tissue increased on day 12 of pregnancy, so also did the rate of basal lipolysis, i.e. tissue + medium FFA content. However, when expressed on a fat cell number basis (Table 13), basal lipolytic rate after day 12 did not differ significantly from virgin controls except on day 3 of lactation. This increase in lipolytic rate on day 3 of lactation was not reflected by increased tissue levels of FFA since a large decrease in FFA content occurred (figure 15).

Thus on day 12 of pregnancy and day 3 of lactation the increased FFA being produced via lipolysis in adipose tissue is release to the medium and may also reflect a low rate of reesterification. During late pregnancy and late lactation the FFA content of adipose tissue

Figure 15. The Changes in FFA Accumulation by Rat Adipose Tissue 'in vitro' during incubation to study Basal Lipolysis during Pregnancy and Lactation.

Details of experiment were as described in text. Triplicate samples were performed on tissue from each animal and the mean of these results was calculated. Each result is the mean (±SEM) of the estimations obtained for all animals on each day studied.

The number of animals studied on each day was 3.


+20
0
-20
-40
-60
% change in accumulation of FFA/120 min.
0
7
12
16
19
3
10
Day of Pregnancy or Lactation.

decreases. This is not reflected by increased release of FFA to the medium (Figure 11), suggesting a more rapid rate of FFA reesterification.

3.3.7. The Changes in FFA Accumulation by Rat Adipose Tissue in vitro during stimulation with Epinephrine during Pregnancy and Lactation.

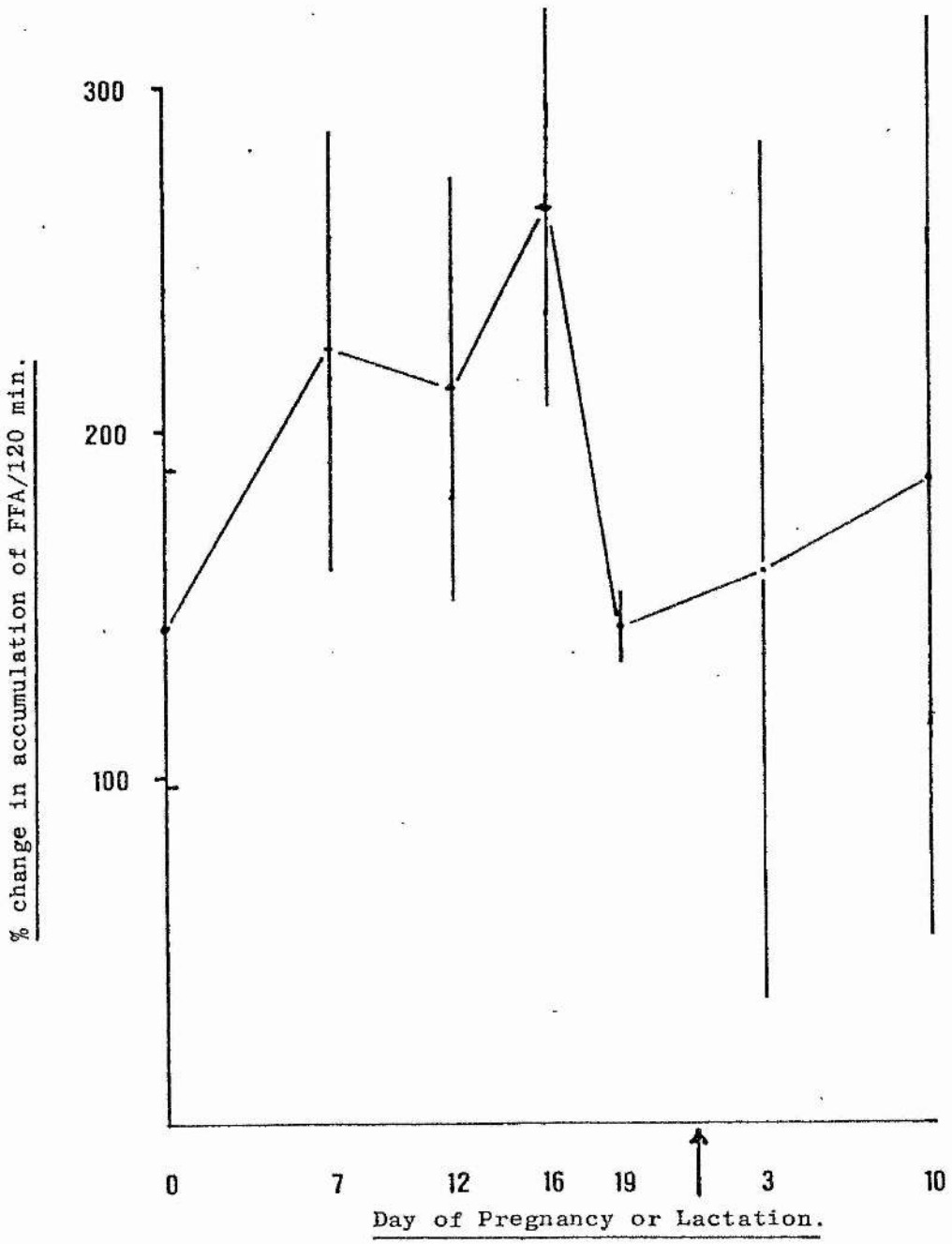
The changes in FFA content of adipose tissue during pregnancy and lactation following incubation with epinephrine is shown in Figure 16.

From the figure it is clear that adipose tissue from virgin, pregnant and lactating rats accumulated FFA during the incubation period. Epinephrine-stimulated FFA release from the tissue to the medium was shown to be increased on days 7 and 12 of pregnancy (Figure 12). Accumulation of FFA in the tissue during a two hour incubation period with epinephrine was increased on these days. This indicates that the rate of reesterification was probably low in tissue from these animals, allowing FFA produced by lipolysis to be released to the medium.

It has clearly been shown (Figure 14) that glycerol production in response to epinephrine does not change significantly between days 16 of pregnancy and 3 of lactation. Hence the marked decline in the build-up of FFA within the tissue during incubation probably results from a relatively small increase in the reesterification capacity. However, the majority of the FFA produced in response to epinephrine is released into the medium at all the stages of pregnancy and lactation.

Figure 16. The Changes in FFA Accumulation by Rat Adipose Tissue 'in vitro' during stimulation with Epinephrine during Pregnancy and Lactation.

Details were as described for figure 15.



On day 10 of lactation, very little of the FFA produced is reesterified; in fact, an unusually high ratio of FFA : glycerol (approx. 4:1; see tables 13 and 15) release is observed.

3.4. Lipolysis in Mouse Parametrial Adipose Tissue

Since a depletion of parametrial adipose tissue lipid content has been reported for the mouse during late pregnancy and lactation (3), it was decided to quantitate T_0 levels of FFA and glycerol and lipolytic rate in mouse adipose tissue in an attempt to correlate this with the histological evidence (3) and as a comparison study with the rat. Tissue FFA content was also quantitated.

Results extend only into early lactation, since insufficient adipose tissue was available in later lactation.

3.4.1. Levels of FFA and Glycerol in Mouse Adipose Tissue at T_0 during Pregnancy and Lactation

FFA and glycerol levels were measured at T_0 in adipose tissue from mice during pregnancy and lactation. This is shown for FFA levels in table 16. The pattern was similar for all methods of result expression. Levels were elevated throughout pregnancy compared with controls and the increase was enhanced during early lactation with a six-fold increase occurring on a fat cell number basis. This pattern was not reflected in glycerol levels (table 17) during pregnancy except on days 13 and 15. Levels of glycerol were elevated in early lactation.

This suggests that although FFA levels are increased, lipolytic stimulation probably does not appear until late pregnancy or early lactation.

Table 16. Levels of FFA in Mouse Adipose Tissue at Zero Time during Pregnancy and Lactation

FFA were estimated as previously described (2.2.5.2). Duplicate estimations were performed on pooled tissue from 3 animals and the mean calculated. Each result is the mean (± SEM) of all estimations on tissue from all animals on each day studied. The number of animals on each day was 9.

	Day of Pregnancy or Lactation					
	virgin	7	13	15	17	2
μmoles/100 mg tissue	0.39 ± 0.05	0.97 ± 0.13	0.69 ± 0.13	0.97 ± 0.04	0.82 ± 0.04	1.51 ± 0.11
μmoles/100 mg lipid	0.46 ± 0.05	1.19 ± 0.28	0.97 ± 0.15	1.36 ± 0.19	1.30 ± 0.04	2.55 ± 0.29
μmoles/10^6 cells	0.91 ± 0.09	1.87 ± 0.21	1.57 ± 0.25	1.67 ± 0.62	1.40 ± 0.53	8.42 ± 1.00

Table 17. Levels of Glycerol in Mouse Adipose Tissue at Zero Time during Pregnancy and Lactation

Glycerol was estimated as previously described (2.2.6.3). Conditions and estimations were as described in Table 16.

	Day of Pregnancy or Lactation					
	virgin	7	13	15	17	2
μmoles/100 mg tissue	0.10 ± 0.01	0.09 ± 0.02	0.15 ± 0.03	0.40 ± 0.25	0.12 ± 0.01	0.17 ± 0.01
μmoles/100 mg lipid	0.12 ± 0.02	0.11 ± 0.03	0.21 ± 0.04	0.56 ± 0.53	0.19 ± 0.01	0.28 ± 0.04
μmoles/10^6 cells	0.23 ± 0.05	0.17 ± 0.01	0.34 ± 0.07	0.69 ± 0.37	0.22 ± 0.06	0.92 ± 0.11

3.4.2. Effect of Pregnancy and Lactation on FFA Release by Mouse Parametrial Adipose Tissue in vitro.

Table 18 shows the release of FFA (Basal lipolysis) by mouse adipose tissue in vitro during pregnancy and lactation.

Basal lipolysis in mouse adipose tissue increased until day 17 of pregnancy when results were expressed on a tissue wet weight basis. By day 2 of lactation the rate had fallen to very low levels.

When the tissue lipid content was used to express results the pattern was similar to that described above except the increase in lipolysis was further enhanced on day 17 of pregnancy. Again release of FFA was very low in early lactation.

In contrast, when results were expressed on a fat cell number basis (Figure 17) basal lipolysis remained essentially unchanged throughout pregnancy except for a small increase on day 13. A very low level of FFA release was obtained in early lactation.

Thus, basal lipolysis appears to increase during pregnancy when results were expressed on tissue wet weight and tissue lipid bases. However, when fat cell number was used for expression of results, no significant change occurred. This again emphasizes the importance of expressing the results on fat cell number. During early lactation the FFA content of tissue and medium was lower than that in the tissue prior to incubation. This suggests either a lower rate of lipolysis during incubation or that the rate of FFA reesterification was increased.

Basal lipolysis in rat and mouse parametrial adipose tissue during pregnancy is fairly similar, showing rates normal or supra-normal during early to mid-pregnancy. However, the rate during

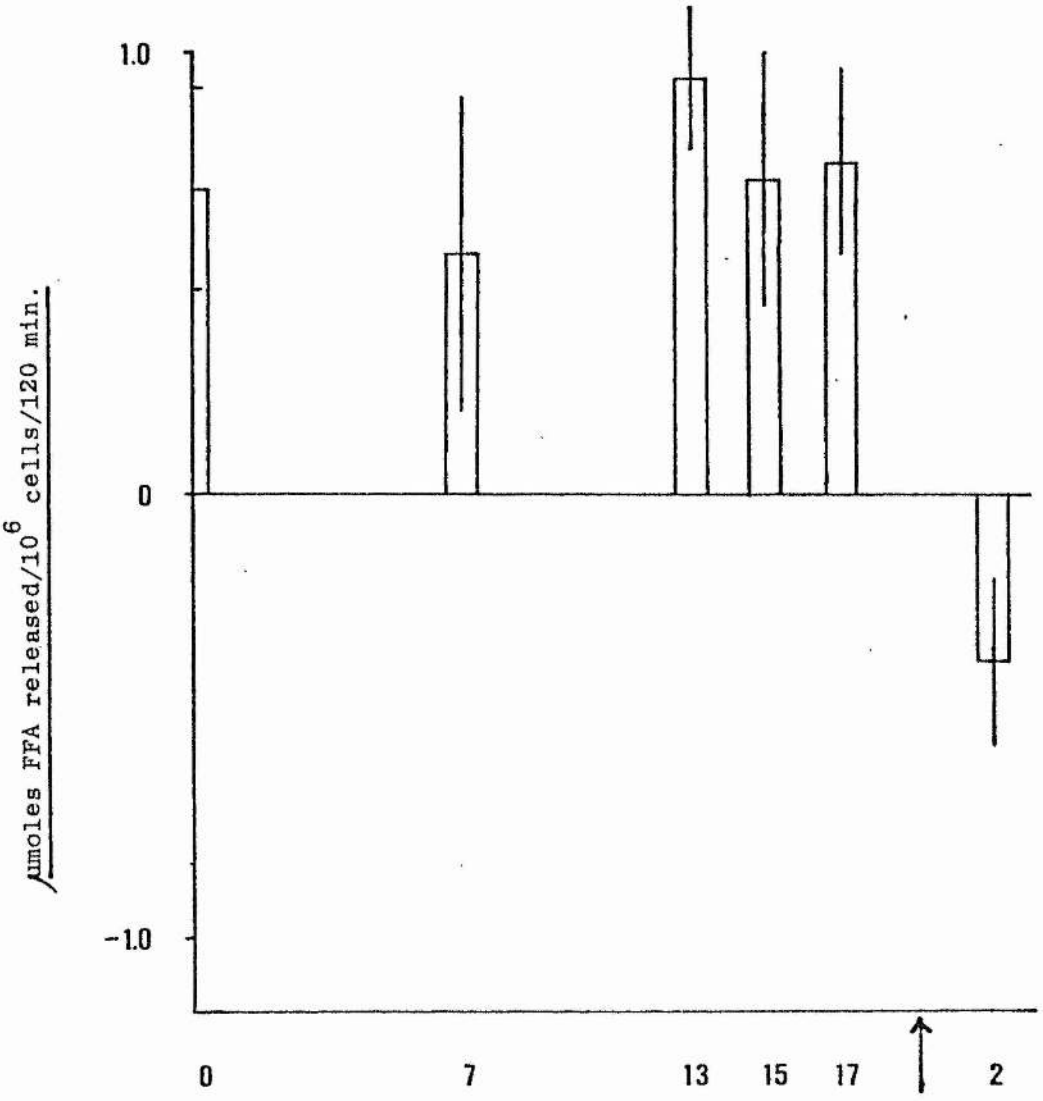
Table 18. The Effect of Pregnancy and Lactation on FFA Release by Mouse Adipose Tissue 'in vitro'.

Conditions of incubations were as described in table 12. Tissue from 3 mice was pooled and triplicate samples performed on the pooled tissue and the mean of these estimations was calculated. Each result is the mean (± SEM) of all estimations on tissue from all animals on each day studied. The number of animals used on each day is shown in parenthesis.

	Day of Pregnancy or Lactation					
	virgin	7	13	15	17	2
µmoles FFA/100 mg tissue/ 120 min.	0.27 ± 0.07	0.29 ± 0.16	0.41 ± 0.06	0.42 ± 0.15	0.48 ± 0.10	-0.12 ± 0.06
µmoles FFA/100 mg lipid/ 120 min.	0.32 ± 0.10	0.35 ± 0.19	0.57 ± 0.08	0.59 ± 0.23	0.76 ± 0.17	-0.21 ± 0.10
µmoles FFA/10^6 cells/ 120 min.	0.68 ± 0.23	0.55 ± 0.36	0.94 ± 0.16	0.72 ± 0.29	0.76 ± 0.21	-0.38 ± 0.19
	(9)	(6)	(9)	(9)	(9)	(9)

Figure 17. The Effect of Pregnancy and Lactation on FFA Release by explants of Mouse Adipose Tissue.

Details of experiment were as described in table 14.



lactation in the mouse was consistently lower than virgin controls whereas the rate in the rat was increased compared with controls.

Therefore, if it is assumed that expressing results on a fat cell number basis is the most valuable method, it appears that basal lipolysis in the mouse during pregnancy does not alter. However, the rate of FFA release is very low during early lactation.

To discover if mouse adipose tissue responds to a lipolytic stimulus during pregnancy and lactation, tissue was incubated in the presence of maximal concentrations of epinephrine and FFA release quantitated.

3.4.3. The Effect of Pregnancy and Lactation on the Response of Mouse Parametrial Adipose Tissue in vitro to Epinephrine Stimulation. Release of FFA.

The response of mouse adipose tissue to epinephrine stimulation during pregnancy and lactation is shown in Table 19.

When results were expressed on tissue wet weight and tissue lipid bases, the rate of epinephrine-stimulated lipolysis increased from virgin controls throughout pregnancy and reached its highest level on day 17. The rate fell slightly, but insignificantly on day 2 postpartum.

In contrast, as already discussed for basal lipolysis, expression of results on a fat cell number basis altered the pattern of epinephrine stimulated lipolysis (Table 19 and Figure 18). No significant change in rate occurred by day 15 of pregnancy, but had increased by day 17. A four-fold increase over virgin control rate occurred on day 2 of lactation.

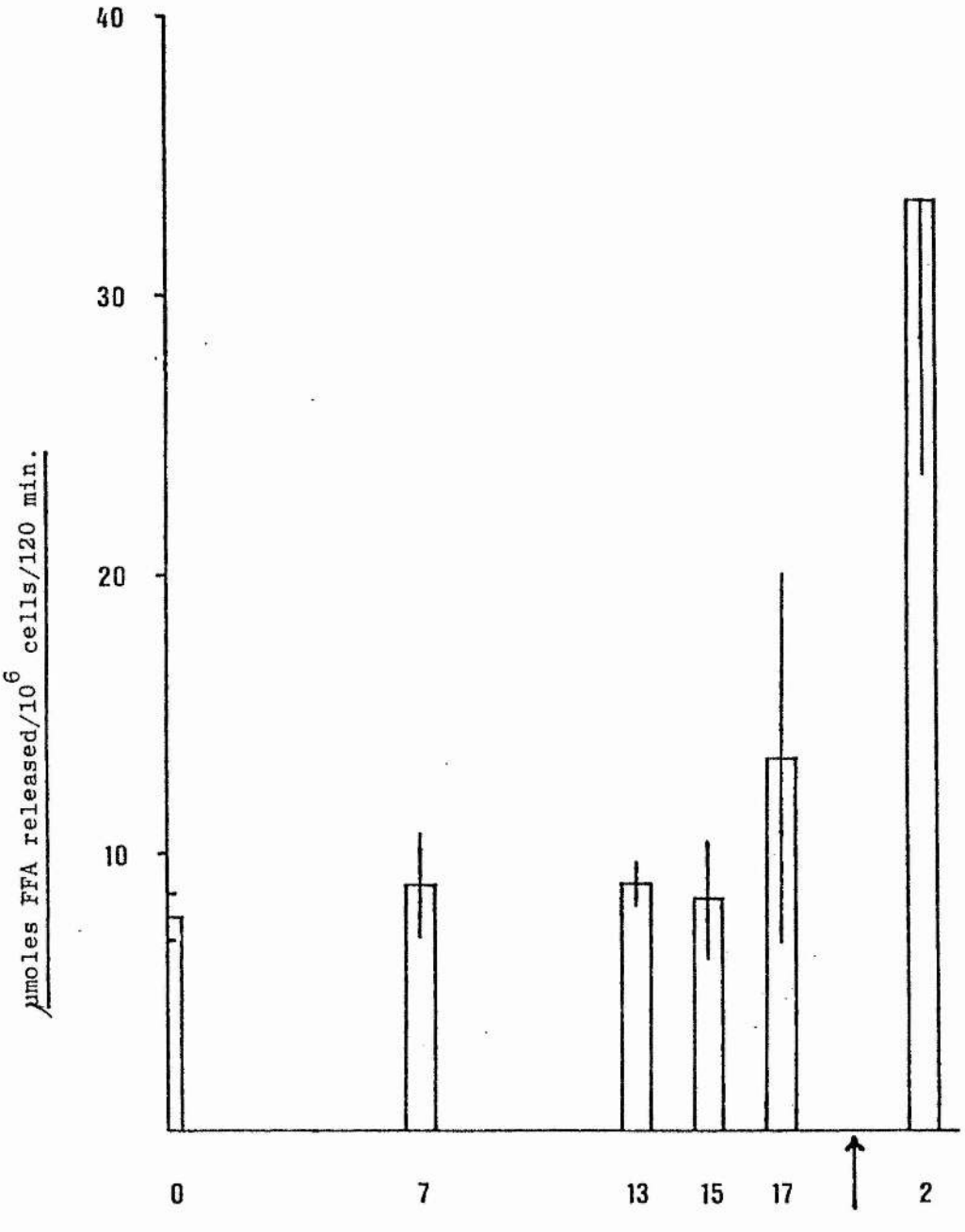
Table 19. The Effect of Pregnancy and Lactation on the Response of Mouse Adipose Tissue to Epinephrine Stimulation 'in vitro'. Release of FFA.

Conditions of incubations were as described in table 12. Tissue from 3 mice was pooled and triplicate samples performed on the pooled tissue and the mean of these estimations was calculated. Each result is the mean (± SEM) of all estimations on tissue from all animals on each day studied. The number of animals used on each day is shown in parenthesis.

	Day of Pregnancy or Lactation					
	virgin	7	13	15	17	2
µmoles FFA/100 mg tissue/ 120 min.	3.23 ± 0.34	4.62 ± 0.26	3.85 ± 0.38	4.90 ± 0.51	7.82 ± 1.66	5.89 ± 0.58
µmoles FFA/100 mg lipid/ 120 min.	3.80 ± 0.40	5.62 ± 0.32	5.46 ± 0.50	6.84 ± 0.57	12.39 ± 4.74	10.12 ± 1.48
µmoles FFA/10^6 cells/ 120 min.	7.69 ± 0.86	8.84 ± 1.90	8.90 ± 0.81	8.41 ± 2.17	13.39 ± 6.70	33.40 ± 5.09
	(9)	(6)	(9)	(9)	(9)	(9)

Figure 18. The Effect of Pregnancy and Lactation on the Response of Mouse Adipose Tissue 'in vitro' to Epinephrine Stimulation. Release of FFA.

Details of experiment were as described in table 15.



It appears that mouse adipose tissue is (i) more sensitive to epinephrine stimulation after day 15 of pregnancy and (ii) shows a higher overall rate of FFA release. Rat adipose tissue shows increased FFA release in response to epinephrine during pregnancy and early lactation. However in contrast to mouse adipose tissue, the response was evident as early as day 7 of pregnancy (Fig. 12).

In summary, mouse adipose tissue exhibits increased response to epinephrine stimulation by FFA release during late pregnancy and lactation but shows no increase in basal lipolysis at any stage of the reproductive cycle.

In the following section, both basal and epinephrine stimulated lipolysis measured by glycerol release in mouse adipose tissue were studied.

3.4.4. The Effect of Pregnancy and Lactation on Glycerol Release by Mouse Adipose Tissue in vitro

Basal release of glycerol from mouse adipose tissue *in vitro* during pregnancy and lactation is shown in Table 20.

Similar patterns of glycerol release were obtained when results were expressed by tissue wet weight or extracted tissue lipid. Mean glycerol release was increased throughout pregnancy and early lactation. During pregnancy, this is generally in agreement with the release of FFA from mouse adipose tissue (Table 18). However, when glycerol release increased during early lactation, FFA release was very low.

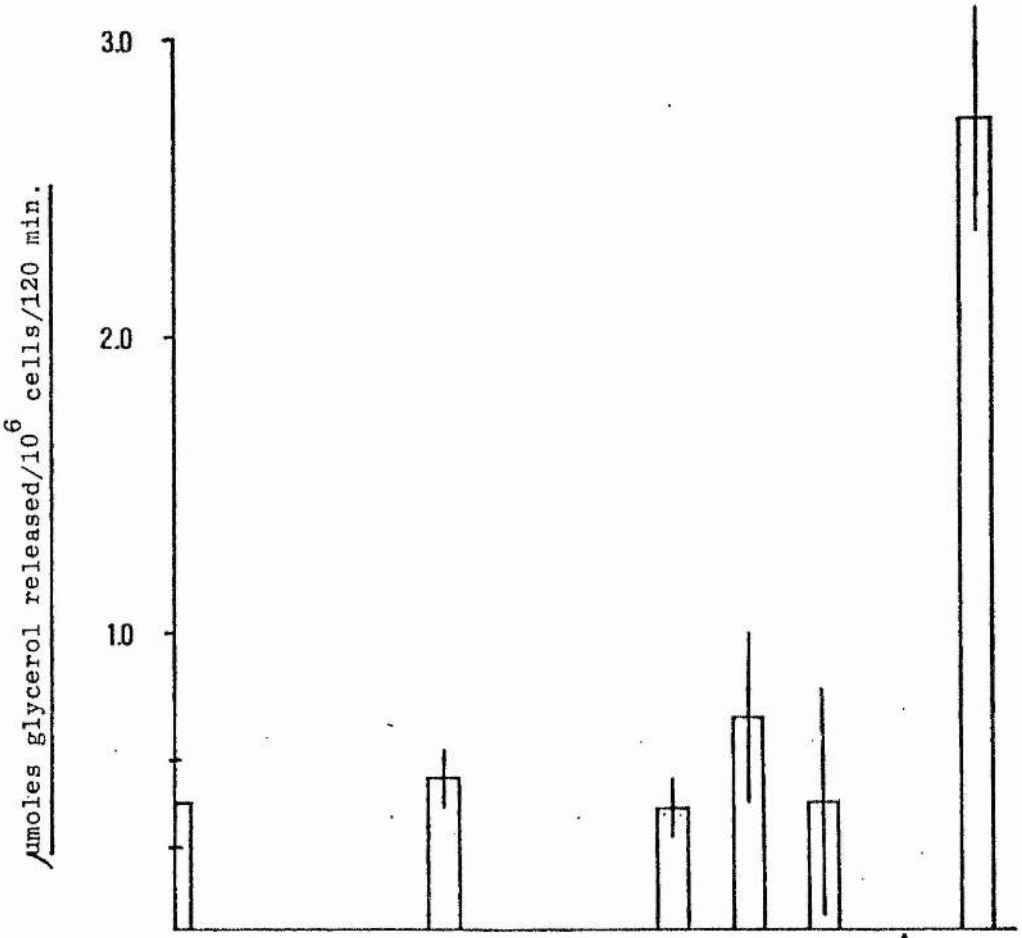
When results were expressed on a fat cell number basis (Table 20 and Figure 19), basal glycerol release was increased insignificantly

Table 20. The Effect of Pregnancy and Lactation on Glycerol Release by Mouse Adipose Tissue 'in vitro'.

Conditions of incubation and estimations were as described in Table 18.

	Day of Pregnancy or Lactation					
	virgin	7	13	15	17	2
µmoles glycerol/100 mg tissue/120 min.	0.18 ± 0.04	0.27 ± 0.03	0.18 ± 0.04	0.41 ± 0.08	0.27 ± 0.09	0.48 ± 0.04
µmoles glycerol/100 mg lipid/120 min.	0.21 ± 0.05	0.33 ± 0.03	0.25 ± 0.04	0.57 ± 0.13	0.43 ± 0.22	0.83 ± 0.11
µmoles glycerol/10^6 cells /120 min.	0.43 ± 0.15	0.51 ± 0.10	0.41 ± 0.10	0.70 ± 0.29	0.44 ± 0.39	2.74 ± 0.31
	(9)	(6)	(9)	(9)	(9)	(9)

Figure 19. The Effect of Pregnancy and Lactation on Glycerol Release by explants of Mouse Adipose Tissue.
Details of experiment were as described in table 16.



on days 7, 15 and 17 of pregnancy. On day 2 of lactation, glycerol release increased almost six fold compared with virgin controls and 17-day pregnant animals. FFA release on day 2, in total contrast, decreased compared with both virgin control and 17-day pregnant animals.

The increase in glycerol release on day 2 of lactation with concomitant decrease in release FFA suggests that the rate of reesterification of FFA is increased in the lactating mouse.

This pattern of glycerol release in mouse adipose tissue agrees with that for the rat during pregnancy (Figure 13). No increase in glycerol release was obvious during late pregnancy in adipose tissue of both species. However, increased release of glycerol is characteristic of early lactation.

3.4.5. The Effect of Pregnancy and Lactation on the Response of Mouse Parametrial Adipose Tissue in vitro to Epinephrine Stimulation. Release of Glycerol

Epinephrine-stimulated glycerol release from mouse adipose tissue in vitro during pregnancy and lactation is shown in Table 21.

When results were expressed on tissue wet weight and tissue lipid bases, although the mean rate of lipolysis changed, e.g. increased rates on day 17 of pregnancy, this was not significant. No significant increase occurred on day 2 of lactation compared with virgin controls when results were expressed on a tissue lipid basis. This is in distinct contrast to the results obtained with FFA release (Table 19), which increased throughout pregnancy and into lactation and may reflect a lower rate of FFA reesterification by mouse adipose tissue. In contrast, when results were expressed on a fat cell

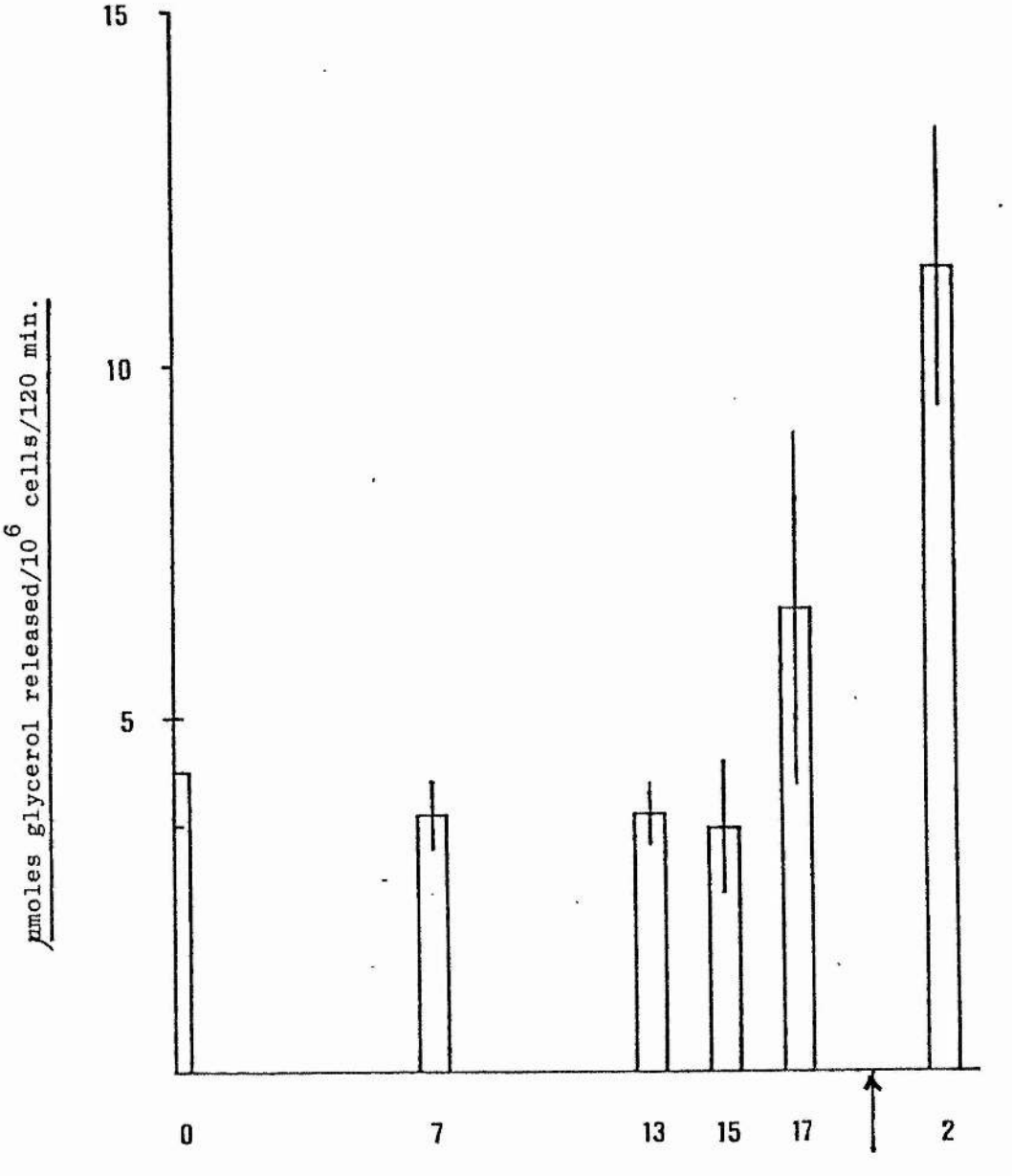
Table 21. The Effect of Pregnancy and Lactation on the Response of Mouse Adipose Tissue to Epinephrine Stimulation 'in vitro'. Release of Glycerol.

Conditions of incubation and estimations were as described in Table 18.

	Day of Pregnancy or Lactation					
	virgin	7	13	15	17	2
µmoles glycerol/100 mg wet weight/120 min.	1.81 ± 0.41	1.96 ± 0.21	1.57 ± 0.21	2.00 ± 0.11	4.02 ± 1.57	2.01 ± 0.22
µmoles glycerol/100 mg lipid/120 min.	2.13 ± 0.48	2.39 ± 0.39	2.22 ± 0.27	2.79 ± 0.13	6.40 ± 3.89	3.45 ± 0.60
µmoles glycerol/10^6 cells/ 120 min.	4.23 ± 0.75	3.62 ± 0.49	3.62 ± 0.46	3.44 ± 0.94	6.53 ± 2.46	11.18 ± 1.51
	(9)	(6)	(9)	(9)	(9)	(9)

Figure 20. The Effect of Pregnancy and Lactation on the Response of Mouse Adipose Tissue 'in vitro' to Epinephrine Stimulation. Release of Glycerol.

Details of eperiment were as described in table 17.



number basis, no significant change in glycerol release occurred until after day 17 of pregnancy when the rate more than doubled compared with controls by day 2 of lactation (Figure 20). This closely correlates with the data for epinephrine-stimulated lipolysis measured by FFA release and confirms that mouse adipose tissue is more sensitive to lipolytic stimulation in early lactation. This is in contrast to the response of rat adipose tissue which shows enhanced sensitivity to a lipolytic stimulus earlier in pregnancy.

As an index of the rate of reutilization of FFA, the change in content of tissue FFA following an incubation period during pregnancy and lactation was also studied.

3.4.6. The Changes in FFA Accumulation by Mouse Adipose Tissue in vitro during Incubation to study Basal Lipolysis during Pregnancy and Lactation

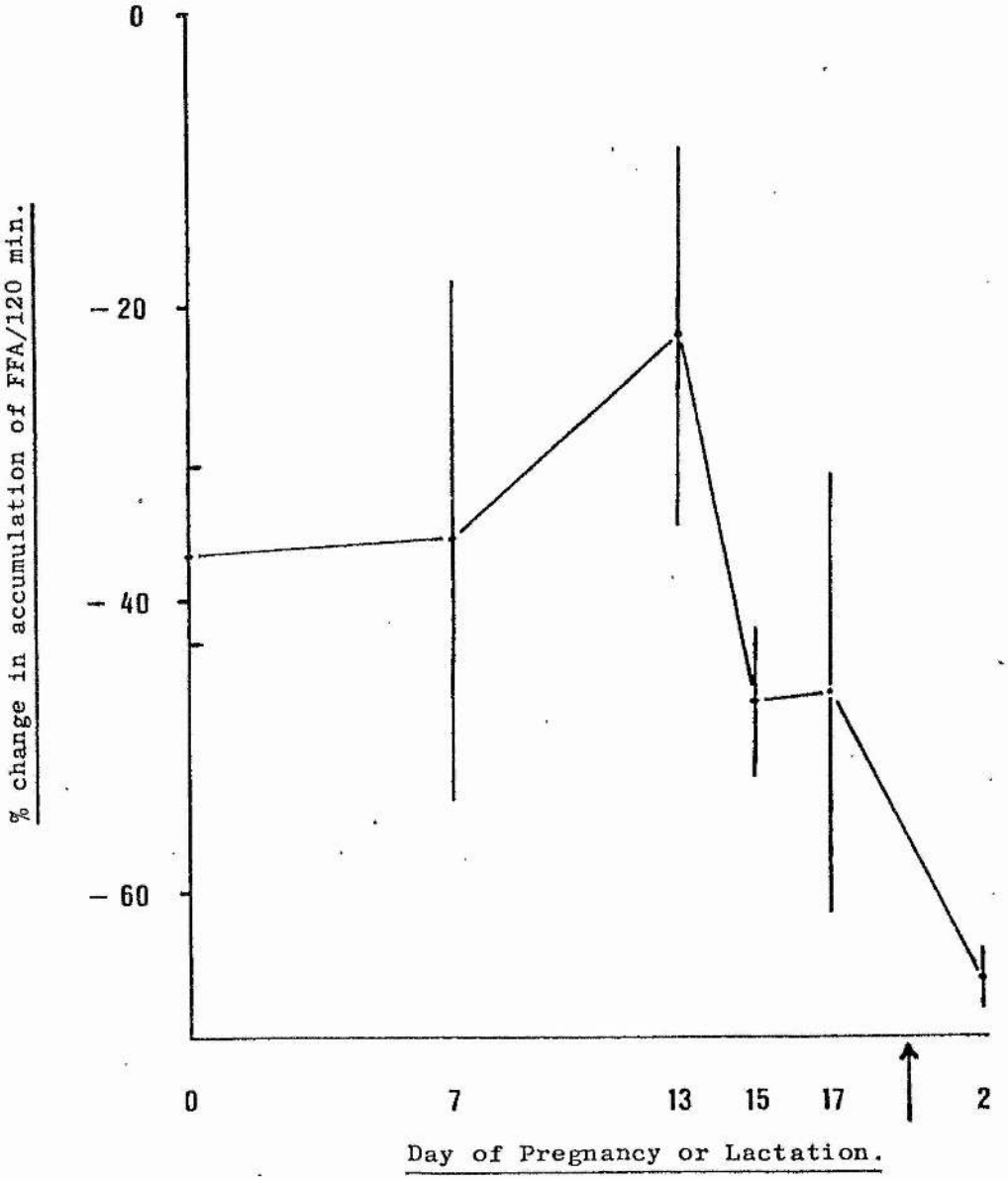
Figure 21 shows the content of FFA in mouse adipose tissue in vitro during pregnancy and lactation relative to initial FFA content following a two hour incubation period. Percent change is based on results expressed by fat cell number.

Two phases of FFA content-change are evident. No significant difference in FFA content was evident until day 13 of pregnancy. Thereafter, FFA content fell rapidly until day 2 of lactation. During incubation, on all days studied FFA were 'lost' by adipose tissue either due to release to the medium or due to reesterification.

The pattern of basal lipolysis in mouse adipose tissue measured by FFA release (Figure 17) and the pattern of tissue FFA content (Figure 21) are very similar. During early- and mid-pregnancy there is no significant change in either the total FFA produced or

Figure 21. The Changes in FFA Accumulation by Mouse Adipose Tissue 'in vitro' during incubation to study basal Lipolysis during Pregnancy and Lactation.

Details of experiment were as described in text. Triplicate samples were performed on pooled tissue from three mice. 9 animals were used on each day except day 7 when 6 were used. Each result is the mean ($\pm$SEM) of all estimations on all tissue from animals used on each day.



in the FFA content of the tissue. However, during late pregnancy and lactation both decrease. Since a loss of FFA from adipose tissue is not reflected by an increase in basal lipolytic rate (i.e. tissue + medium FFA), it appears that the tissue increases its rate of FFA reesterification. The rate of basal lipolysis in mouse adipose tissue, measured by glycerol release, increases during early lactation (Figure 19). This confirms that increased FFA re-esterification does take place during early lactation in mouse adipose tissue.

3.4.7. The Changes in FFA Content of Mouse Adipose Tissue in vitro (during Epinephrine Stimulation) during Pregnancy and Lactation

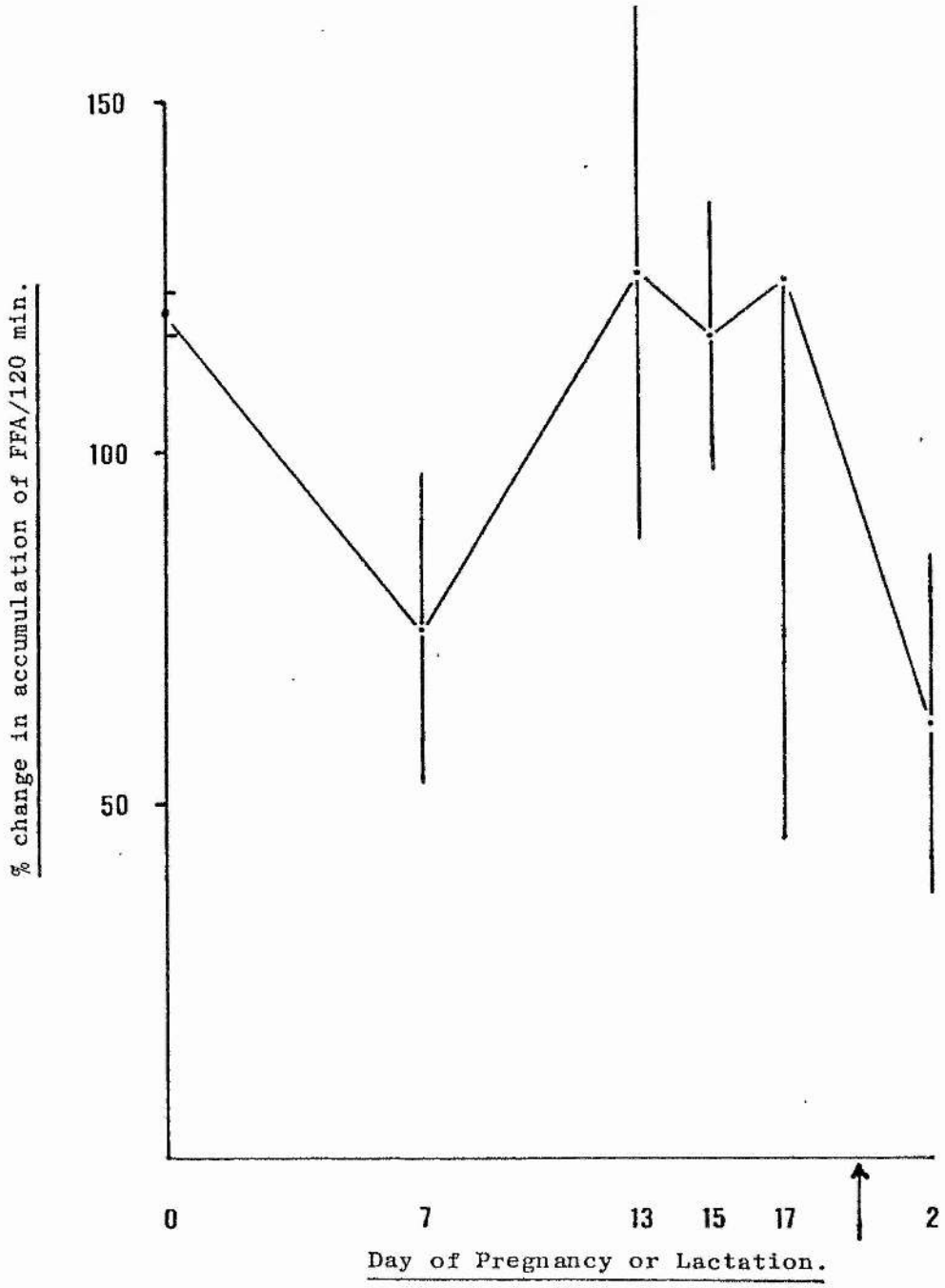
The changes in FFA content of mouse adipose tissue in vitro during pregnancy and lactation in response to epinephrine stimulation are shown in Figure 22.

It is clear that in contrast to FFA content during basal lipolysis, adipose tissue FFA content increased on all days of pregnancy and lactation in response to epinephrine stimulation.

The reduced extent of FFA accumulation during the incubation period using tissue from mice pregnant 7 days and lactating 2 days may result from (i) an increased release of FFA from the cell to the medium or, (ii) a decreased net production of FFA via hydrolysis of intracellular triglyceride. Since glycerol release is elevated in late pregnancy and early lactation (Figure 20), it is most probably the former explanation which applies.

Figure 22. The Changes in FFA Content of Mouse Adipose Tissue 'in vitro' during Epinephrine simulation during Pregnancy and Lactation.

Details were as described for figure 21.



3.4.8. Summary of Lipolysis in Rat and Mouse Parametrial Adipose Tissue

Basal lipolysis measured by FFA release in the rat is insignificantly increased in early- and mid-pregnancy and increased in early lactation, but was not different from controls on other days. Release of FFA in response to epinephrine stimulation is increased during pregnancy and early lactation. The rate decreases later in lactation.

Basal lipolysis measured by glycerol release in rat adipose tissue is not significantly different from virgin controls excepting increases on day 12 of pregnancy and early lactation. Epinephrine-stimulated glycerol release is elevated during mid- and late pregnancy and early lactation. The rate of release of glycerol in later lactation is decreased.

The rate of FFA reesterification in rat adipose tissue is increased during late pregnancy.

In mouse adipose tissue basal lipolysis measured by FFA release is not significantly different from virgin controls throughout pregnancy but decreases in early lactation. Epinephrine-stimulated lipolysis increases in late pregnancy and further increases in early lactation.

Basal and epinephrine-stimulated lipolysis measured by glycerol release increases in early lactation. Epinephrine-stimulated lipolysis is also increased in late pregnancy.

FFA reesterification rate is increased in late pregnancy and early lactation during basal lipolysis in mouse adipose tissue.

3.5. The Effect of Hormones on the Activity of Rat Parametrial Adipose Tissue Lipoprotein Lipase in vivo

The control of the changes in adipose tissue carbohydrate and lipid metabolism during pregnancy and lactation is probably hormonal and it is thus important in trying to formulate control mechanisms to delineate the effects of individual hormones. Since it has been shown that lipoprotein lipase activity decreases in adipose tissue in late pregnancy and lactation (Figure 9) and increases in mammary gland (57) the control mechanism may be similar but with conflicting effects on the two tissues. It was therefore decided to study the control of adipose tissue lipoprotein lipase activity in more depth by the administration of hormones *in vivo* to virgin animals.

In deciding which hormones should be used in the study several factors were considered. Firstly, which of the hormones involved in the mammary gland lactogenic trigger may also be involved in the regulation of lipoprotein lipase activity? Secondly, published data on the blood levels of these hormones were correlated with the lipoprotein lipase activity changes noted *in vitro* (section 3.2.2). Finally, possible hormone interactions were also considered.

The main lactogenic hormone, prolactin, was thought to be the most likely factor in lipoprotein lipase control. Prolactin has already been reported to affect lipoprotein lipase activity in rat mammary gland and adipose tissue *in vivo* (48) and in pigeon adipose tissue *in vivo* (128). Oestrogen is also involved in milk production in rat mammary gland and has been reported to affect adipose tissue lipoprotein lipase activity (51-53). The blood levels of both of these hormones also increase during late pregnancy (plate 1) and

therefore may be involved in the control of lipoprotein lipase *in vivo*. The effect of a combined injection regimen on lipoprotein lipase activity *in vivo* has not been studied.

The mechanism of action of prolactin and oestrogen in regulating lipoprotein lipase activity has not been studied. This is of interest since oestrogen can induce the release of rat prolactin *in vivo* (117). It is possible that oestrogen affects lipoprotein lipase activity by promoting prolactin release. The prolactin blocker, α-ergocryptine, was therefore used in the study.

Therefore, prolactin, oestrogen and α-ergocryptine injections were employed in the following *in vivo* experiments.

Figure 23 shows the effect of hormone and α-ergocryptine administration on virgin rat adipose tissue lipoprotein lipase activity *in vivo*

i. Injection of pure corn-oil and saline subcutaneously into control animals

Oestrogen and α-ergocryptine were injected in pure corn-oil. Control animals received corn-oil subcutaneously. This resulted in an increase in lipoprotein lipase activity compared with virgin, non-injected controls. This was most likely due to release of oil into the general circulation resulting in induction of lipoprotein lipase activity in adipose tissue. A similar effect has been demonstrated in rats tube-fed with corn-oil (129).

Prolactin was injected in 0.9% NaCl and the control animals also received 0.9% NaCl.

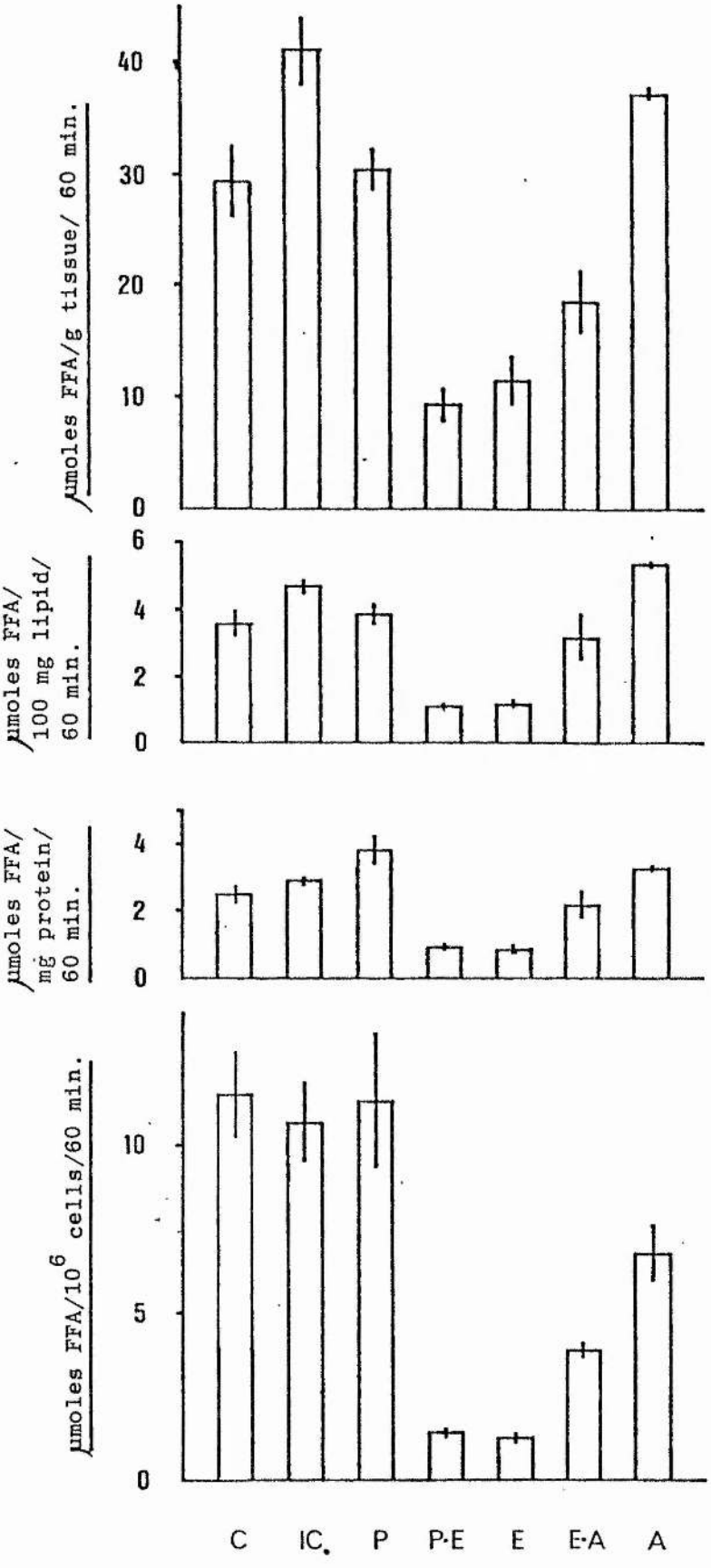
Comparisons were therefore made with corn-oil/saline injected animals as controls.

Figure 23. The Effect of Hormones and ∝-Ergocryptine on the Activity of Rat Adipose Tissue Lipoprotein Lipase.

Details of the injection schedules are described in the text.
Each result is the mean (±SEM) of all determinations on tissue of all animals receiving a particular injection regimen.
Significance tests were performed using the Students t-test.

Abbreviations;
C : non-injected controls
IC : injected controls
P : prolactin-injected
E : oestradiol benzoate-injected
A : ∝-ergocryptine-injected.
P.E : prolactin + oestradiol benzoate-injected
E.A : oestradiol benzoate + ∝-ergocryptine-injected

Significance values.



IC v P : 0.05
IC v P.E : 0.001
IC v E : 0.001
IC v A : 0.05
IC v E.A : 0.02
E v E.A : NS

IC v P : NS
IC v P.E : 0.01
IC v E : 0.001
IC v A : NS
IC v E.A : NS
E v E.A : NS

IC v P : NS
IC v P.E : 0.001
IC v E : 0.001
IC v A : NS
IC v E.A : NS
E v E.A : NS

IC v P : NS
IC v P.E : 0.02
IC v E : 0.02
IC v A : NS
IC v E.A : 0.05
E V A : 0.01

ii. Effect of Prolactin on the activity of rat adipose tissue lipoprotein lipase in vivo

Animals received, daily, 0.5mg Prolactin in 0.5ml saline and 0.2ml corn-oil on 7 consecutive days.

A significant but small decrease in lipase activity was obtained with prolactin administration when results were expressed by tissue wet weight. However, when tissue lipid, extracted protein and fat-cell number were used to express results, no significant difference in enzyme activity occurred.

Prolactin at this concentration does not appear to affect the activity of adipose tissue lipoprotein lipase.

iii. Effect of Prolactin plus Oestrogen on the activity of lipoprotein lipase in rat adipose tissue in vivo

Animals received 0.5mg of prolactin and 5µg of oestrogen/day for 7 days.

The combined regimen of prolactin and oestrogen was found to produce a significant decrease in lipoprotein lipase activity in vivo.

Oestrogen may therefore be responsible for a drop in enzyme activity. However, it may be that oestrogen induces prolactin release from the pituitary which, combined with the injected prolactin, affects enzyme activity.

iv. Effect of Oestrogen on the activity of lipoprotein lipase in rat adipose tissue in vivo.

To investigate the oestrogen effect further, oestrogen (5µg/day in corn-oil) was injected for 7 days along with 0.5ml saline.

Administration of oestrogen caused a significant decrease in lipase activity. This activity was not significantly different from the activity caused by the combined prolactin and oestrogen

treatment. It would appear that oestrogen either produces its effect on the enzyme by a direct effect on adipose tissue or through prolactin release from the pituitary.

v. Effect of Oestrogen plus α-ergocryptine and α-ergocryptine alone on the activity of lipoprotein lipase in rat adipose tissue in vivo.

To discover if oestrogen exerts its effect on lipoprotein lipase activity by release of prolactin, α-ergocryptine was used to block prolactin release, during the administration of oestrogen.

Animals received 0.5mg of α-ergocryptine (this has been shown to block oestrogen-induced prolactin release (118)) and 5µg of oestrogen/day for 7 days.

Administration of α-ergocryptine with saline did not produce a significant difference in lipoprotein lipase activity.

Injection of oestrogen and α-ergocryptine produced a significant decrease in lipase activity when results were expressed by tissue wet weight and fat cell number. The decrease in activity was not significant when extracted tissue lipid and extract protein were used to express results.

These results are rather conflicting but it does appear that oestrogen does have a direct effect on enzyme activity, or at least the effect is not via modification of prolactin release.

Summary

i. Injection of corn-oil alone induced increased LPL activity in rat adipose tissue.

ii. Prolactin (0.5mg/day) did not decrease LPL activity significantly in adipose tissue.

iii. Oestradiol benzoate decreased LPL activity significantly in adipose tissue.

iv. Oestradiol benzoate + prolactin decreased LPL activity significantly in adipose tissue, but this decrease did not exceed that produced by oestradiol benzoate alone.

v. α-Ergocryptine did not produce a significant decrease in LPL activity in adipose tissue.

vi. Oestradiol benzoate + α-ergocryptine decreased lipase activity in adipose tissue but not always significantly.

vii. Oestradiol benzoate + α-ergocryptine did not cause a significant increase in LPL activity on a tissue wet weight basis in adipose tissue but did on other methods of expression of results compared with oestradiol benzoate alone.

On the evidence presented here it appears that oestrogen has the ability to decrease the activity of adipose tissue lipoprotein lipase in the virgin rat. The method of action of the hormone is not known but it may have some direct effect on adipose tissue. Oestradiol increases in late pregnancy and may be responsible for the fall in enzyme activity at this time in normal pregnant animals. Further work is necessary to elucidate the physiological significance of this result.

In contrast, virgin rat adipose tissue is insensitive to the action of prolactin at the concentration used. It may be that a higher concentration would elicit a response in the tissue or that the virgin rat requires pre-sensitization before it responds to prolactin, i.e. hormonal sensitization during late pregnancy.

3.6. Production of Antiserum against Bovine Prolactin

Although adipose tissue from virgin rats did not respond to the action of prolactin, it is possible that prolactin, the main lactogenic hormone, is responsible for the decreased levels of activity of adipose tissue lipoprotein lipase in lactating rats. It was decided that by neutralizing prolactin during lactation, and hence reducing or stopping milk production, any effect of the hormone on adipose tissue lipoprotein lipase activity would also be reversed. If prolactin reduced enzyme activity, a rise in activity following hormone neutralization would be expected.

It was hoped to raise antibodies to bovine prolactin in rabbits, to characterize this antiserum against rat prolactin, and to inject the antiserum into lactating rats to neutralize prolactin.

Characterization of Antiserum.

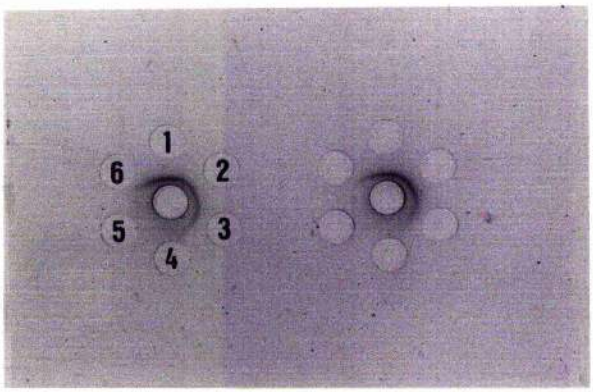
3.6.1. Double-diffusion of Antiserum to Bovine Prolactin against Bovine Prolactin.

Plate 2A shows the results of Ouchterlony double-diffusion studies with antiserum from the first rabbit used. Dilution of prolactin up to 1:160 was used. Antiserum was undiluted.

Plate 2. Double-diffusion of Antiserum to Bovine Prolactin against Bovine Prolactin.

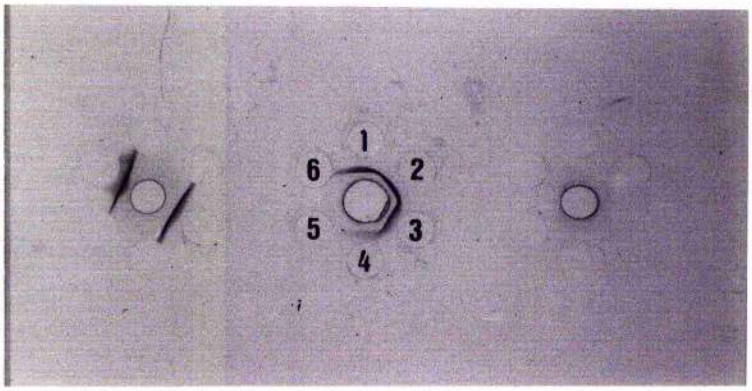
Experimental conditions were as described in the text. Dilutions were performed on an initial prolactin solution of 1mg/ml.

A. Antiserum raised using Freunds Incomplete Adjuvant.



Well N°.	Dilution.
1	undiluted
2	1:10
3	1:20
4	1:40
5	1:80
6	1:160

B. Antiserum raised using Freunds Complete Adjuvant.



Well N°.	Dilution.
1	1:20
2	1:40
3	1:80
4	1:160
5	1:320
6	1:640

A precipitation line was obvious confirming the presence of antibodies in the rabbit serum. An antiserum : prolactin precipitate was obtained to a prolactin dilution of 1 : 40.

It was decided that this may not be a high enough antibody titre. Another rabbit was injected using Freund's complete adjuvant in an attempt to increase the titre.

3.6.2. Double-diffusion of Antiserum to Bovine Prolactin raised using Freund's Complete Adjuvant against Bovine Prolactin

Plate 2B shows the result of Ouchterlony double-diffusion studies using this antiserum and prolactin dilutions of 1 : 20 to 1 : 640. Undiluted antiserum was used.

Precipitation lines were obvious up to prolactin dilutions of 1 : 80. The titre had therefore been increased by the use of Freund's complete adjuvant.

3.6.3. The Tanned-cell Technique to Obtain a Titre of Prolactin Antiserum

The tanned-red cell technique was also used to obtain an antiserum titre. A titre was obvious by the cells settling to a coating in the bottom of wells in the dish. If no reaction occurred, a small compact button was observed.

Controls, i.e. uncoated cells + antiserum (1:10) and coated cells + saline both gave buttons.

Doubling dilutions of antiserum to 1 : 2560 were made and mixed with tanned and coated cells. A coating of cells, indicating reaction between cells and antiserum was obtained only to dilutions of between 1 : 40 and 1 : 80. This therefore confirms the titre obtained in gels.

3.6.4. Effect of Injection of Prolactin Antiserum into Adult Lactating Rats on Litter Weight Gain

Rats received 0.5ml antiserum intraperitoneally daily. Controls received 0.5ml of 0.9% NaCl intraperitoneally daily.

Figure 24 shows the effect of injecting mothers with prolactin antiserum daily during lactation. No obvious loss of ability of the rats to nurse the pups was observed since the litter weights continued to increase.

It would be unlikely for adipose tissue lipoprotein lipase activity to be affected and this therefore was not studied. It was decided that the antiserum titre was not high enough to neutralize the prolactin in the blood of lactating rats. Not enough time was available to use different methods, i.e. different routes of injection of rabbits, to try to raise the titre.

Since the above method was unsuccessful, another means of neutralization of prolactin was attempted. The specific prolactin blocker, α-ergocryptine, which prevents prolactin release from the pituitary, was used. α-Ergocryptine was injected into lactating rats and the litter weight gain followed as an index of prolactin neutralization. It was hoped that, if litter weight gain was reduced, the activity of lipoprotein lipase in adipose tissue would correspondingly increase.

3.6.5. Effect of α-Ergocryptine Injections into Adult Lactating Rats on Litter Weight Gain

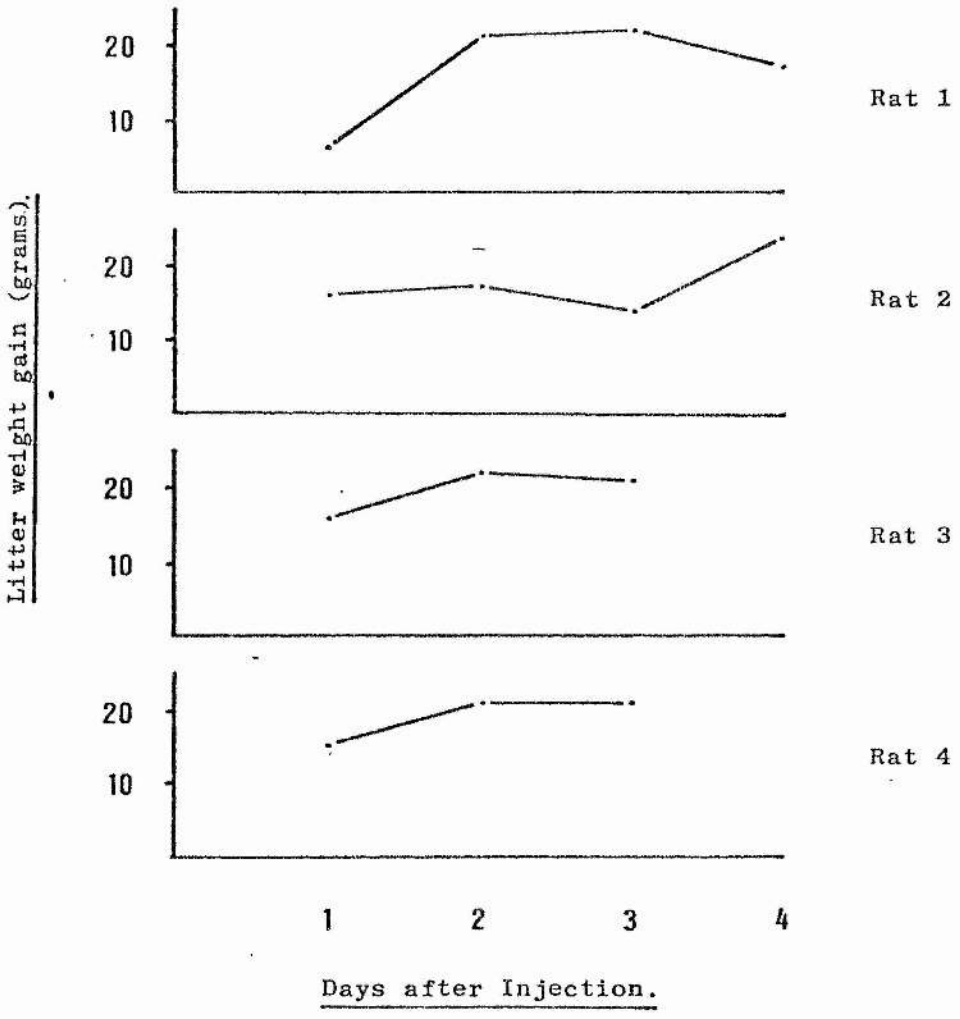
Rats received either 0.5ml of 10% ethanol in 0.9% NaCl or 1mg of α-ergocryptine in 0.5ml of 10% ethanol in 0.9% NaCl intraperitoneally. Litters were adjusted to 10 pups per rat.

Figure 24. Effect of Injection of Prolactin Antiserum into Adult Rats on Litter Weight Gain.

Details of experiment were as described in the text. Litters were weighed each day. The number of pups in each litter was 10.

Rats 1 & 2 : antiserum-injected

Rats 3 & 4 : saline-injected.



Figures 25 and 26 show the weight gain of each litter and the weight of the adult rat during lactation.

The litter of rats injected with saline on day 3 of lactation continued to gain weight after the injection until day 6 of lactation. Nursing mothers continued to gain weight following injection of saline.

The litter of all rats gained weight increasingly until day 3 of lactation. Following α-ergocryptine injection, weight gain was much reduced. Adults also continued to gain weight following drug injection.

These results show that α-ergocryptine probably blocked release of prolactin, since milk synthesis was much reduced in animals which received the drug. It was decided to study the activity of lipoprotein lipase in adipose tissue at the time when milk production was reduced.

3.6.6. Effect of α-Ergocryptine on the Activity of Lipoprotein Lipase in Adipose Tissue in vivo in Lactating Rats

Figure 27 shows the effect of α-ergocryptine on lipoprotein lipase activity in adipose tissue.

In all cases of result expression, α-ergocryptin caused a significant increase in lipoprotein lipase activity. This increase brought the lipase activity in adipose tissue to levels very similar to those in injected control animals (Figure 23).

Since ergocryptine is a toxic material, causing vasoconstriction, (153), caution must be taken in interpreting the results presented here. However, the result of vasoconstriction may be to reduce

Figure 25. The Effect of Saline or ∝-Ergocryptine Injections on Litter Weight Gain.

Details of experiment were as described in the text. Each point is the mean (±half the range) of the weight gain of three litters.

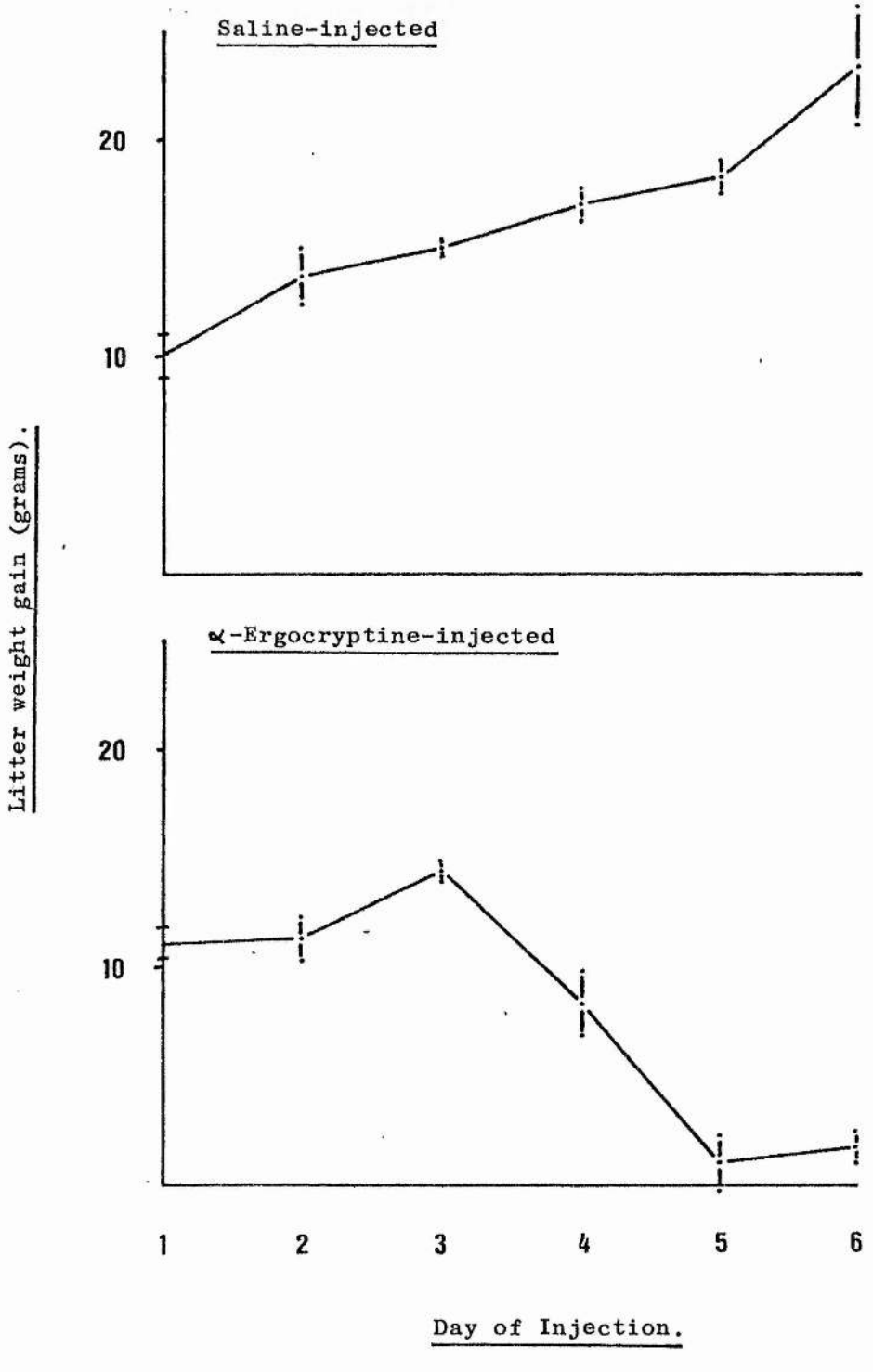


Figure 26. The Effect of Saline or ∝-Ergocryptine Injections on Adult Rat Weight.

Details of experiment were as described in the text. Each point is the mean (± half the range) of the weight of three rats receiving the same injection regimen.

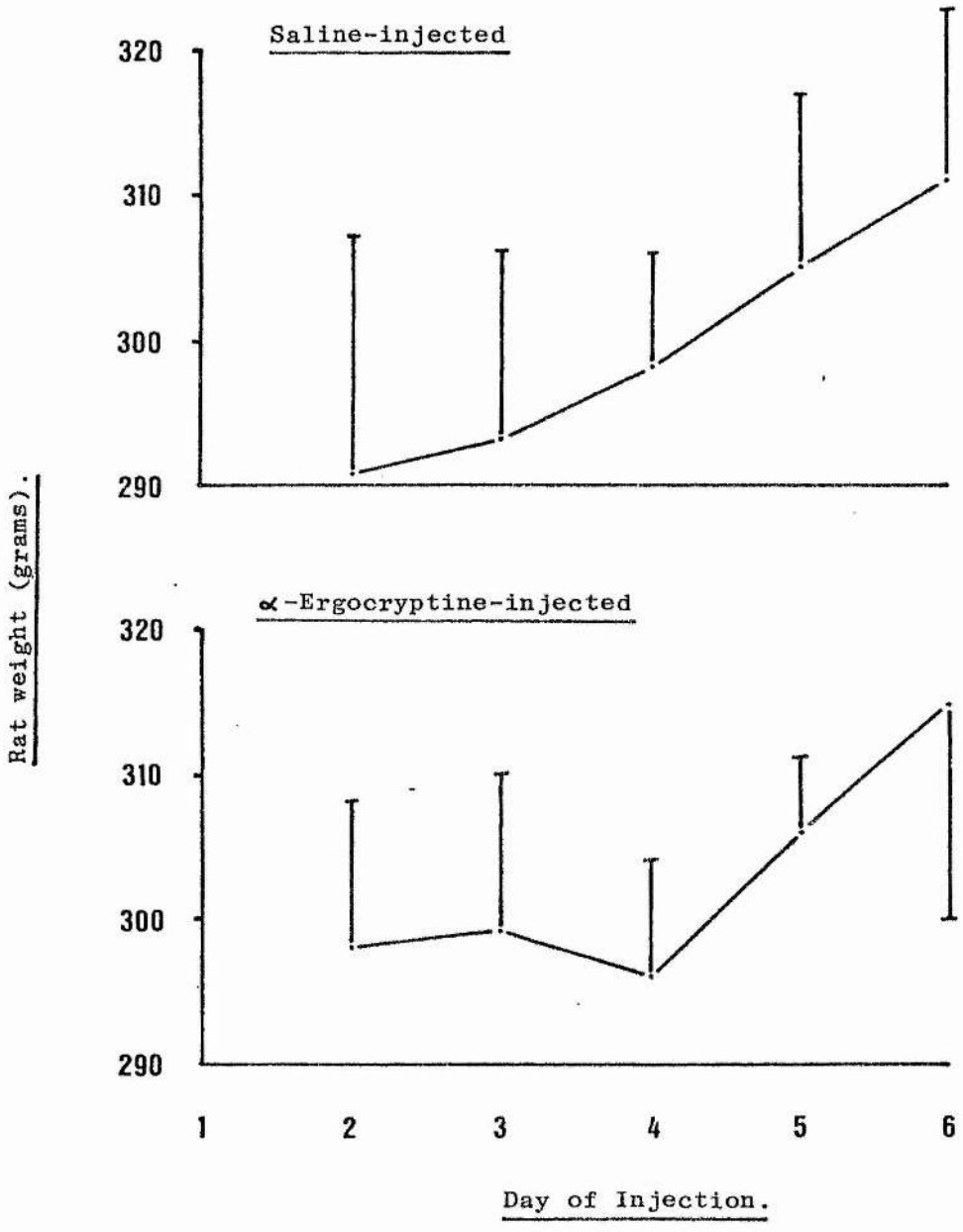
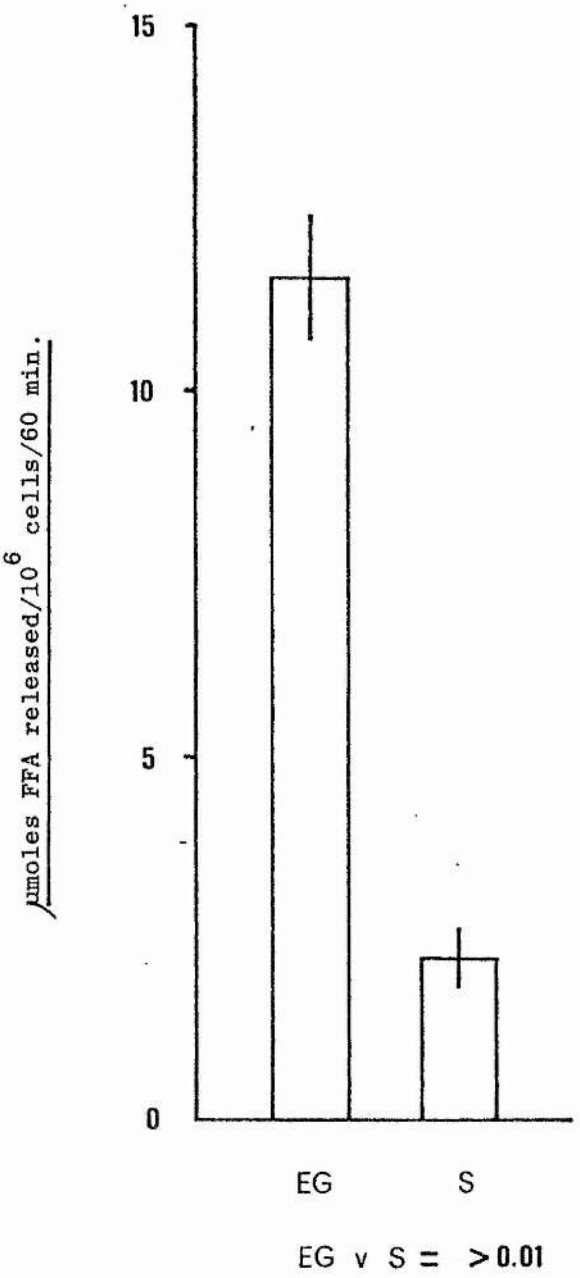


Figure 27. The Effect of α-Ergocryptine Injection into Lactating Rats on the Activity of Adipose Tissue Lipoprotein Lipase.

Details of experiment were as described in the text.
Each result is the mean (±SEM) of triplicate determinations on tissue from three rats.
Significance test was performed using the Students t-test.



EG : α-ergocryptine-injected

S : saline injected.

availability of substrate/inducer and hence enzyme activity. A toxic effect of α-ergocryptine would not, presumably, result in an increase in enzyme activity and so the increase in activity must be due to decreased prolactin release caused by α-ergocryptine.

It appears, then, from these results that prolactin may be the factor which controls the activity of lipoprotein lipase in adipose tissue during lactation, reducing the activity, and so lipid uptake, at a time when lipid uptake by mammary gland is at a maximum. It may be that another factor(s) is required to bring about the fall in enzyme activity during pregnancy, but that this level is maintained by the action of prolactin during lactation. Since oestradiol benzoate induces a decrease in lipoprotein lipase in the virgin rat (Figure 23), oestradiol may be the factor which induces the fall in activity in the pregnant rat. This will be discussed in the following section.

4. DISCUSSION

4. DISCUSSION

Glucose oxidation and fatty acid synthesis rates were quantitated in rat parametrial adipose tissue *in vitro* during pregnancy and lactation.

Rat parametrial adipose tissue *in vitro* exhibited increased capacity for glucose oxidation during early- and mid-pregnancy, a three-fold increase occurring by day 12 of lactation (Figure 7). On all days of pregnancy studied until at least day 16, glucose oxidation was either normal or supra-normal.

During late pregnancy and lactation, oxidation of glucose by rat adipose tissue *in vitro* proceeded at a very low rate, seven-fold lower on day 8 of lactation than virgin controls. This decrease in glucose oxidation rate by adipose tissue is clearly unexpected in view of the increased food intake during late pregnancy in the rat. The decline in adipose tissue oxidative activity is consistent with maximum growth of the foetal unit during late pregnancy (4) and initiation of milk synthesis in the mammary glands (130). Glucose uptake by foetuses and mammary glands increases at this time and may result in decreased availability of glucose to adipose tissue. Plasma glucose concentration in the rat is, indeed, depressed on day 16 of pregnancy and continues to fall until the end of pregnancy (4).

Results presented here (Figure 7) confirm earlier findings (24) that oxidation of 6-^{14}C-glucose by rat adipose tissue *in vitro* is increased on days 7 and 16 of pregnancy. However, no fall in

oxidation rate later in pregnancy was reported by Smith (24).

Comparison of Figures 7 and 8 reveals that the patterns of glucose oxidation and fatty acid synthesis from glucose in adipose tissue *in vitro* were very similar. During early- and mid-pregnancy, fatty acid synthesis was either elevated or not significantly different from virgin controls. Thus, the increased flow of glucose through glycolysis was reflected by utilization of carbohydrate precursors in increased fatty acid synthesis. This in turn would release $NADP^+$ for the reduction steps of the pentose phosphate pathway and increase the flow of glucose through the pathway to produce three-carbon precursors for *sn*-glycerophosphate formation. This and the fattyacyl CoA molecules produced, would result in increased synthesis and storage of triglyceride in adipose tissue.

Increased fatty acid synthesis by rat adipose tissue both *in vitro* and *in vivo* has also been reported on days 12 and 16 of pregnancy (4,6).

During late pregnancy and lactation, the rate of fatty acid synthesis fell below virgin control levels until it was eight-fold lower by day 8 of lactation. This is clearly consistent with the depressed rate of glucose utilization at this time. This is in conflict with the work of Knopp *et al.* (4) who showed that fatty acid synthesis remained elevated in adipose tissue from 19-day pregnant rats and did not fall until day 21. This anomaly may reflect an animal strain difference or housing conditions. Fatty acid synthesis from ^3H_2O increased on day 16 compared with day 20 of pregnancy in rat adipose tissue *in vivo* (6). The rate on day 20 was comparable to that of virgin controls. There is, therefore

some conflicting evidence as to the timing of the fall in fatty acid synthesis in adipose tissue during pregnancy. However, it does appear to fall after day 16 and possibly before day 19 of pregnancy.

Depressed levels of fatty acid synthesis during lactation in rat adipose tissue in vitro were also found by Smith (24).

Evidence, therefore suggests that there is biphasic activity of glucose oxidation and fatty acid synthesis rates by rat adipose tissue during pregnancy, as suggested by Knopp (4). Early- and mid-pregnancy are characterized by elevated levels of glucose oxidation and lipid synthesis from glucose, followed by decreased levels in late pregnancy. This results in increased deposition of lipid in adipose tissue. The depressed levels are maintained during lactation when, probably, little de novo synthesis and storage of lipid occurs. Thus, adipose tissue has effectively released glucose for utilization by other tissues, e.g. the foetal unit which increases fatty acid synthesis from $^{3}H_2O$ on day 20 compared with day 16 (6) and the mammary glands (130).

The mechanism of the fall in glucose utilization and fatty acid synthesis is not known. It may simply be due to increased uptake of glucose by the foetal unit and mammary glands, reducing the availability of glucose to adipose tissue, as previously discussed. This explanation is unlikely, however, since the increased food intake during the second half of pregnancy may compensate for the increased utilization by foetal unit and mammary glands.

Since glucose transport into adipose tissue is controlled by insulin (13), it is reasonable to assume that impairment of insulin

action is responsible for the fall in glucose utilization. Insulin is thought to regulate the activities of the glycolytic pathway enzymes hexokinase and pyruvate dehydrogenase and also the enzyme acetyl CoA carboxylase (21, 23). Antagonism of insulin action would result in decreased transport of glucose into adipose tissue and flow through glycolysis. Plasma insulin increases during the third trimester of pregnancy (4) and, therefore, tissue-selective antagonism of insulin action is necessary to explain the differences in glucose utilization rates, for example, between adipose tissue and the mammary glands. The mammary glands also require insulin for full development prior to milk synthesis in vitro (101, 102). More work is necessary on the oxidative activity of other tissues during pregnancy and lactation to explain this.

A possible mechanism whereby adipose tissue becomes insensitive to the action of insulin has been suggested (4). It is thought that progesterone or chorionic somatomammotropin may be the insulin antagonist. Plasma progesterone concentration reaches a peak during the second half of pregnancy and may reach critical levels for insulin antagonism in adipose tissue. Chorionic somatomammotropin may produce insulin antagonism by its reported lipolytic action (131). Lipolytic agents which raise cAMP levels in adipocytes decrease glucose transport and, conversely, anti-lipolytic agents in decreasing cAMP levels, increase glucose transport (132). Human placental lactogen has lipolytic activity (133) and it is possible that the rat placental lactogen, produced from day 10 of pregnancy, may also exhibit this characteristic, thus antagonizing glucose transport.

Of the hormones which increase their concentrations in serum during late pregnancy (plate 1) none seems capable of depressing glucose utilization in adipose tissue or very little is known about their effects on the tissue. Prolactin increases glucose utilization in adipose tissue and can, therefore, be discounted. Further work is required on the effect of the ovarian hormones and corticosteroids on the tissue.

Both adipose tissue and lactating mammary gland utilize blood lipid (36). The rate of uptake is determined by the prevailing physiological conditions and, more directly, by the activity of lipoprotein lipase. The activity of this enzyme provides a convenient tool for the study of lipid uptake in tissues. If blood lipid is to be used in milk synthesis, it would be expected that lipid storage in adipose tissue would be reduced and, therefore, so would the activity of lipoprotein lipase.

The activity of lipoprotein lipase in rat adipose tissue falls during early-pregnancy, consistent with earlier findings (56, 57), and may be due to the hormonal changes which occur following fertilization and implantation. The enzyme activity recovers after day 5 until, by mid-pregnancy, it is similar to that of virgin controls (Figure 9). Otway and Robinson (56) also reported levels in mid-pregnancy to be similar to virgin controls, while Hamosh et al. (57) found lipase activity at this stage to greatly exceed control levels.

It appears, then, that adipose tissue maintains or increases lipid uptake during mid-pregnancy, if it is assumed that lipoprotein lipase activity reflects lipid uptake. In view of the increased synthesis of lipid shown earlier (Figure 8), and the increased

uptake, it is probable that deposition of triglyceride in adipose tissue is elevated during pregnancy in the rat, confirming earlier findings that the rat increases its body fat content during gestation (5).

Late-pregnancy is characterized by low levels of activity of lipoprotein lipase and these are maintained until at least day 8 of lactation. This has also been shown in earlier studies (56, 57). Indeed, Hamosh et al. (57) have shown that the low level of activity of lipase is maintained until day 15 of lactation.

Lipid uptake and deposition by adipose tissue is, therefore, much reduced during late-pregnancy and lactation allowing blood lipid to be used preferentially by tissues other than adipose which are more active at this time. Mammary gland lipoprotein lipase activity does, in fact, increase during late gestation and lactation (57), and this is true for a number of species (36).

Thus there are two phases of lipid metabolism in rat adipose tissue during pregnancy and lactation. In general, lipid synthesis and uptake are maintained or increased during early- and mid-pregnancy resulting in elevated triglyceride stores. These stores may then be used at a time when there is strain on the animal's energy reserves, e.g. during lactation. The second phase is characterized by low levels of fat uptake, synthesis and subsequent deposition.

Control of lipoprotein lipase activity during pregnancy and lactation is probably hormonal. However, control may be achieved by substrate availability, since tube-feeding rats with triglyceride induces lipoprotein lipase activity in adipose tissue in vivo (129). Blood triglycerides increase throughout gestation in the rat (57)

and, therefore, substrate induction may explain the recovery of activity in mid-pregnancy. However, blood triglycerides continue to increase in late pregnancy and this is clearly inconsistent with the decrease in lipoprotein lipase activity in adipose tissue (Figure 9).

Glucose induces lipoprotein lipase activity in adipose tissue in vitro (46), and omission of glucose from the incubation medium results in decreased lipase activity in the medium (49). The fall in lipase activity during pregnancy coincides with the fall in rat plasma glucose concentration and may partly explain the enzyme activity decrease. During diabetes and starvation, when glucose availability to adipose tissue is decreased, lipase activity is also decreased (30-33). This, however, is thought to be due to the lack of insulin in the diabetic and lack of glucose-induced insulin in the starved animal. A high serum glucose concentration did not increase lipoprotein lipase activity in adipose tissue from starved rats in the absence of insulin, and injected insulin prevented a fall in enzyme activity when plasma glucose concentration was falling (50). Thus it is thought that both glucose and insulin are necessary to induce lipoprotein lipase activity in adipose tissue from starved rats. It is possible that insulin is necessary for glucose entry into adipose tissue and this in turn induces enzyme activity.

As suggested for lipid synthesis, insensitivity to insulin action may partly explain the decrease in lipoprotein lipase activity in adipose tissue during pregnancy. Patten (134) suggests a mechanism whereby activation of lipolysis may result in decreased lipoprotein lipase activity in adipocytes in vitro. Incubation of

adipocytes with the omission of glucose and insulin results in a 50% fall in lipoprotein lipase activity in the cells. This fall is abolished by addition of glucose and insulin. Inhibition of protein synthesis by addition of cycloheximide prevented the rise induced by glucose and insulin and led to decay of lipase activity. It was also found that lipolytic concentrations of dibutyryl cAMP added with glucose and insulin decreased the activity of lipoprotein lipase as well as the rate of protein synthesis. Patten suggests that dibutyryl cAMP decreases the ATP content of adipocytes by increasing the process of lipolysis and subsequent reesterification of FFA. Thus the availability of ATP for protein synthesis falls and, therefore, synthesis of lipoprotein lipase. Close correlation between lipoprotein lipase activity and protein synthesis was found.

Lipolytic hormones, by increasing the content of cAMP in rat adipocytes, decrease glucose transport (132). Insulin no longer increases glucose transport when cAMP is raised by these hormones. Thus, by blocking glucose transport and protein synthesis in adipose tissue, lipolytic hormones may be important in controlling lipoprotein lipase activity. This is also an effective regulatory mechanism for adipose tissue, in that FFA uptake is decreased at a time when the animal requires FFA mobilization from adipose tissue, mediated by lipolytic stimulators.

Oestrogen is considered to be a lipaemic hormone and there is a close correlation between plasma oestradiol and the increase in blood triglycerides during pregnancy in women (135). Could oestradiol be the inhibiting factor in the control of lipoprotein lipase activity during pregnancy?

Oestradiol benzoate injection produced a significant decrease in adipose tissue lipoprotein lipase activity in fed virgin rats (Figure 23). No further fall was induced by a combined injection regimen of oestradiol benzoate plus prolactin, nor was the decrease completely corrected by administration of a dose of α-ergocryptine which is known to block oestradiol-stimulated prolactin release from the rat pituitary in vivo (118). Oestradiol, therefore, either has a direct effect on lipase activity in adipose tissue, or an effect not wholly mediated via prolactin release. The inhibitory effect of oestrogens on lipoprotein lipase activity in adipose tissue has also been shown elsewhere (51-53).

Alternatively, oestradiol may act through its possible action of $PGF_{2\alpha}$ release from the pregnant rat uterus (136, 137). Indeed, premature delivery induced by oestradiol in ovariectomized rats could be overcome by the addition of a prostaglandin-release blocker (136). It is suggested that a pituitary factor, possible LH, induces oestradiol release from the ovary and the oestradiol induces $PGF_{2\alpha}$ from the uterus. There is considerable evidence that $PGF_{2\alpha}$ is luteolytic (97, 137-139) and its release may be the factor involved in progesterone withdrawal during late pregnancy. It has been suggested that progesterone withdrawal is the mechanism which induces the decrease in lipase activity in adipose tissue (54). Simultaneous injection of oestradiol benzoate and the prostaglandin blocker, indomethacin, may be of interest in this field..

Evidence for the effect of progesterone on lipoprotein lipase activity in adipose tissue is conflicting. Hamosh and Hamosh (51) reported that progesterone had no effect on adipose tissue lipoprotein lipase activity from either male or ovariectomized rats.

whereas Kim and Kalkhoff (53) showed that progesterone increased lipase activity in adipose tissue from fed female rats. More recently, Spooner (54) has shown that progesterone increases adipose tissue lipase activity and that the fall in lipase activity in 20-day pregnant rats induced by $PGF_{2\alpha}$ injection is due to progesterone withdrawal as a result of the luteolytic effect of $PGF_{2\alpha}$ (54). $PGF_{2\alpha}$ injection reduced serum progesterone by 90%. However, foetal corticosteroids may also play an important role in progesterone withdrawal (140, 141). This mechanism does not explain the fall in lipase activity in adipose tissue reported here (Figure 9), since the fall in enzyme activity occurred prior to the withdrawal of progesterone during pregnancy (plate 1).

The fall in adipose tissue lipoprotein lipase activity induced by oestradiol benzoate was partly corrected by simultaneous α-ergocryptin administration, suggesting that oestradiol benzoate may act partly via release of prolactin from the pituitary (Figure 23). However, prolactin injection at the concentration used did not produce a significant change in adipose tissue lipoprotein lipase activity in vivo in virgin rats (Figure 23). Injections of prolactin into rats pregnant 16 and 20 days did not induce a significant change in adipose tissue or mammary gland activity (54). From this evidence it can be considered that prolactin is not the factor which causes the decrease in lipoprotein lipase activity in adipose tissue. However, injection of prolactin into hypophysectomized lactating rats increased mammary gland lipoprotein lipase activity and blocked the return of activity in adipose tissue (48). Prolactin alone, therefore, may not have the capacity to induce changes in adipose tissue lipoprotein lipase activity but may be capable of maintaining the low activity produced in hypophysectomized animals.

This argument can, perhaps, be extended to the lactating condition. Prolactin serum concentration increases only very late in pregnancy and remains high throughout lactation (plate 1), and it is possible that prolactin is involved in the maintenance of the low activity of lipoprotein lipase characteristic of lactation. To study this, a specific antiserum was raised to prolactin in rabbits for injection into lactating rats in an attempt to neutralize the high circulating level of prolactin. Unfortunately as reported in the Results section, the titre of this antiserum proved to be too low and the prolactin -release blocker, α-ergocryptine, was used instead. This proved to be most effective in blocking prolactin release as evidenced by the decrease in weight gain of the litters following drug injection (Figure 25). Control litters continued to gain weight.

The low level of lipoprotein lipase activity in adipose tissue during lactation was reversed when α-ergocryptine was administered to lactating animals, the activity being similar to that of virgin controls. Lactating animals not receiving α-ergocryptine maintained a low lipase activity. This suggests that, although prolactin alone is not capable of inducing a fall in lipoprotein lipase activity in adipose tissue in pregnant rats, it may be involved in the maintenance of low activity during lactation. Since the lipase activity in drug-injected rats was similar to the controls, it is possible that prolactin alone maintains the low activity.

Rat placenta produces a lactogenic hormone (rat placental lactogen) during the second half of pregnancy (88, 142). Since prolactin release increases only very late in pregnancy and does not affect lipoprotein lipase activity in the virgin or pregnant rat,

rat placental lactogen may be involved in the control of lipase activity during pregnancy. Further work is required to study this, possibly involving injections into rats of placental extracts from animals at various stages of gestation.

Clearly, the changes which occur in lipoprotein lipase activity in adipose tissue in the rat during late pregnancy are not the result of the action of a single factor: not surprising in view of the complex changes in hormone levels. The fall in lipase activity in adipose tissue may be due to any one or all of the following factors:

i. insulin insensitivity of adipose tissue during the third trimester due to rat placental lactogen and/or progesterone.

ii. increased lipolysis causing decreased glucose transport and protein synthesis.

iii. increased levels of oestradiol.

iv. production of $PGF_{2\alpha}$ by the uterus inhibiting release of progesterone, possibly mediated through LH and oestradiol.

v. increased production of rat placental lactogen converting progesterone to its hydroxy derivative.

Prolactin may, as discussed, be the hormone which controls lipoprotein lipase activity in adipose tissue during lactation.

The mechanism of action of hormones controlling lipoprotein lipase activity has not yet been fully elucidated. It may be by direct action by interfering with the enzyme-membrane connection at the luminal surface of the endothelial cell as proposed by Olivecrona, et al. (143). Alternatively, hormones may act via the adipocyte nucleus controlling synthesis of the enzyme.

It is known that the serum apoproteins C are involved in the activation of lipoprotein lipase (42). Reports suggest that different classes of apoprotein C may activate and inhibit the enzyme: CII apoprotein activating and CIII apoprotein inhibiting (144). Recently, it was shown that the ratio of CII : CIII apoproteins in sera of women in the third trimester of pregnancy decreases. This is, therefore, another possible control mechanism for lipoprotein lipase during pregnancy.

Since adipose tissue may contribute to milk production by decreasing synthesis and uptake of lipid during late pregnancy and lactation, does it contribute more directly by mobilizing stored triglyceride?

A large (approximately 100%) insignificant increase in basal FFA release by adipose tissue occurred on days 7 and 12 of pregnancy (figure 11). However, glycerol release was elevated only on day 12. The increased mean rate of FFA release may result from decreased re-esterification due to competition for sn-glycerol-3-phosphate between the FFA formed by lipolysis and that by de novo synthesis. Synthesis is elevated at this stage in gestation. It appears that the turnover of lipids in adipose tissue is increased during early- to mid-pregnancy.

Significantly, there was increased release of both FFA and glycerol on day 3 of lactation suggesting that the tissue had been subjected to lipolytic stimulation at this time. Further evidence for this is increased T_0 levels of FFA and glycerol on day 3 of lactation (Tables 10, 11).

To determine if rat adipose tissue became more sensitive to a lipolytic stimulus during pregnancy or lactation, tissue was subjected to incubation with epinephrine. The response of the tissue, in both

FFA and glycerol release, was increased between day 12 of pregnancy and day 3 of lactation. FFA release was also elevated on day 7 of pregnancy, but since there was no rise in glycerol release, reesterification was probably decreased. The fall in the response of the tissue on day 10 of lactation is somewhat surprising, if adipose tissue is producing FFA for milk synthesis. There is evidence to suggest that small fat cells respond less to epinephrine than do larger cells (124, 125). The cells from adipose tissue of 10 day pregnant rats were smaller than on any other day studied and this may explain the diminished response. However, increased lipolytic response has been shown as late as day 14 of lactation (7), and clearly more work remains to be done.

Thus, there is evidence of increased lipolytic activity in rat adipose tissue in vivo on only two days during pregnancy and lactation, i.e. day 12 of pregnancy and day 3 of lactation. This is based solely on basal glycerol release since FFA release is complicated by varying rates of reesterification. There is evidence of decreased rates of reesterification during early pregnancy and increased rates on day 19 of pregnancy (Figures 11 and 13). Tissue on day 19 of pregnancy and day 3 of lactation showed a net loss of FFA during incubation (Figure 15). Since FFA release fell between days 16 and 19 of pregnancy (Figure 11), FFA reesterification is probably the explanation of the net loss. However, net loss coincides with increased release between day 19 of pregnancy and day 3 of lactation (Figure 11), suggesting little reesterification. Quantitatively, the decrease in FFA accumulation by the tissue was similar to the changes in release from the tissue and therefore FFA

reesterification may contribute significantly to the control of basal FFA efflux from the fat cell.

Adipose tissue showed increased response to lipolytic stimulation throughout pregnancy (Figure 13), with evidence of increased rates of FFA reesterification. However, reesterification accounted for less than 10% of the total FFA released, and thus contributed much less to control of release in this instance.

In summary, it has been shown that rat adipose tissue showed increased capacity to respond to a lipolytic stimulus throughout pregnancy, but this potential was not realized in vivo as shown by basal lipolytic rates. Only on day 12 of pregnancy and day 3 of lactation did basal lipolysis appear to be increased. It is interesting to note that rat placental lactogen reaches a peak in serum concentration on day 12 of pregnancy (142) and may induce the increased lipolytic response. Human placental lactogen exhibits lipolytic activity (131) and this may also be the case for the rat hormone.

Evidence from other sources also suggests lipid mobilization during pregnancy and lactation. Increased lipolysis by adipose tissue from fed 19-day pregnant rats was demonstrated by increased production of FFA and glycerol in the presence of glucose (25). This report also showed increased rates of FFA reesterification in pregnant animals. Elliot (8) observed elevated basal lipolysis in fat cells of late-pregnant women. Lipolysis in response to epinephrine was elevated in adipocytes from late pregnant and lactating women, being highest in late-pregnancy. Smith (7) demonstrated increased basal and epinephrine-stimulated lipolysis in rat adipose tissue in vitro during peak lactation, compared with unmated controls.

The blood supply to the reproductive organs, and hence to parametrial adipose tissue is important in discussing the changes in lipid metabolism during pregnancy and lactation. The blood supply is increased during late-pregnancy and early-lactation (145, 146). This cannot be responsible for the decrease in lipid synthesis and uptake in adipose tissue since increased blood flow would merely increase substrate availability. However, increased removal of FFA from the extracellular space of adipose tissue by increased blood flow may relieve TG-lipase from product inhibition (147).

There is little information available concerning adipose tissue lipid metabolism during pregnancy and lactation in the mouse and the results presented in this thesis provide a comparison with those for the rat.

Basal lipolysis in mouse adipose tissue, measured by both FFA and glycerol release did not differ signficiantly from controls during pregnancy (Figures 17 and 19). Glycerol release during early lactation increased markedly, but no rise in FFA release was evident. Thus, it appears that lactation in the mouse is accompanied by increased lipolysis and reesterification of FFA in adipose tissue.

When adipose tissue was exposed to lipolytic stimulation it was evident that an increase in sensitivity did not occur until late pregnancy. Response to epinephrine in FFA and glycerol release was greatly elevated in early lactation.

In the case of basal lipolysis, the rat and the mouse appear to operate similar systems, with increased efflux of FFA and glycerol only significantly elevated during lactation. The rates of re-esterification differ, however, with a period during early pregnancy

of decreased reesterification in the rat which is not evident in the mouse. FFA reesterification was more marked, however, in mouse adipose tissue during lactation.

Increased sensitivity of the tissue to epinephrine in the mouse occurs only toward the end of pregnancy, in contrast to that of the rat. Thus the mouse appears to be incapable of mobilizing stored adipose tissue lipid until late pregnancy.

This is in agreement with histological evidence (3) which shows that the parametrial fat pads of the mouse are depeleted of lipid during late pregnancy and lactation.

Lipoprotein lipase activity in mouse adipose tissue showed a different pattern to that of the rat. Enzyme activity decreased during early-pregnancy, but no mid-pregnancy rise in activity was evident. It is possible that the mouse protects its adipose tissue stores by maintaining normal response to lipolytic factors or conditions throughout pregnancy, since lipid uptake is probably depressed during pregnancy. Measurement of the lipid synthetic rate in mouse adipose tissue during pregnancy and lactation is necessary to elucidate this point.

There was a rise in lipoprotein lipase activity in late-pregnancy and this activity was maintained in early-lactation. The fall in enzyme activity was approximately 50% while rat lipoprotein lipase activity, at a similar stage in lactation, fell to approximately 25% of controls. This increase in lipase activity may be a mechanism whereby mouse adipose tissue repletes its adipose tissue lipid stores during the rapid mobilization of lactation. Lipid turnover in the mouse during early lactation appears to be higher than that of the rat.

Little work has been done to elucidate hormonal control mechanisms for lipoprotein lipase activity during mouse pregnancy. Ovarian activity is necessary throughout gestation for maintenance of pregnancy (147), and this, combined with increased PRL release during pregnancy (148), may be sufficient to lower the activity of lipoprotein lipase.

CONCLUSION

It appears that the rat is perfectly poised for lactation. Stores of lipid are increased during early- and mid-pregnancy in preparation for the stress of lactation. Indeed, lipid uptake and synthesis are decreased during late pregnancy and lactation, thus rerouting substrates to mammary gland or other tissues. Adipose tissue is also more sensitive to lipolytic stimulation during pregnancy and early lactation and there is evidence of mobilization of adipose tissue triglyceride during early lactation.

The mouse also reduces the level of lipid uptake in adipose tissue during pregnancy. In contrast to the rat, there is no evidence of a build-up of lipid stores during pregnancy. This may explain the small amount of adipose tissue in mice during very late pregnancy. The increase in lipoprotein lipase activity on day 17 of pregnancy may be the mechanism by which adipose tissue stores are gradually repleted. Mouse adipose tissue, unlike rat adipose tissue, shows an enhanced response to lipolytic stimulation only very late in pregnancy and the increased sensitivity is maintained into early lactation.

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